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Evaluation of a recirculating pond system for rearing juvenile freshwater mussels at White Sulphur Springs National Fish Hatchery, West Virginia, U.S.A. **ANDREA MUMMERT, TAMMY J. NEWCOMB, RICHARD J. NEVES, and BRUCE PARKER** 1

Disappearance of a population of pygmy octopus following a harmful algal bloom in a northwestern Florida bay, U.S.A. **BRIDGET N. TIFFANY, NANN A. FANGUE, and WAYNE A. BENNETT** 11

A new species of *Sonorella* (Pulmonata: Helminthoglyptidae) from Arizona, with notes on predation and evasive behaviors. **LANCE H. GILBERTSON and WILLIAM R. RADKE** 17

Indicators of physiological condition in juveniles of *Utterbackia imbecillis* (Bivalvia: Unionidae): A comparison of rearing techniques. **GINGER R. FISHER and RONALD V. DIMOCK, JR.** 23

Freshwater molluscs of Fort Stewart, Georgia, U.S.A. **KATHRYN E. SUKKESTAD, EUGENE P. KEFERL, and THOMAS D. BRYCE** 31

Observations on a cohort of the cut-ribbed ark, *Anadara floridana* (Conrad, 1869), from coastal Georgia. **ALAN J. POWER and RANDAL L. WALKER** 39

Seasonal growth and mortality of juveniles of *Lampsilis fasciola* (Bivalvia: Unionidae) released to a fish hatchery raceway. **SHANE D. HANLON and RICHARD J. NEVES** 45

continued on back cover

Cover photo: Shell of *Daedalochila peregrina* from Coles & Walsh

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Seasonal growth and mortality of juveniles of *Lampsilis fasciola* (Bivalvia: Unionidae) released to a fish hatchery raceway. **SHANE D. HANLON and RICHARD J. NEVES** 45

Strategies for sustainable dye harvest of the purple conch *Plicopurpura pansa* (Gould, 1853) from west central Mexico. **ERNESTO A. CHÁVEZ and JESÚS E. MICHEL-MORFÍN** 51

The freshwater gastropods of Iowa (1821-1998): Species composition, geographic distributions, and conservation concerns. **TIMOTHY W. STEWART** 59

Experimental studies on habitat preference and tolerances of three species of snails from the Snake River of southern Idaho, U.S.A. **STEVEN LYSNE and PETER KOETSIER** 77

Effects of extracts of the bark of the stem of *Croton tiglium* on the metabolism of the freshwater gastropod *Lymnaea acuminata.* **RAM P. YADAV, D. SINGH, S. K. SINGH, and A. SINGH** 87

Histology of selected regions of the alimentary system of *Strombus gigas* Linnaeus, 1758 (Caenogastropoda: Strombidae). **OMAR H. AVILA-POVEDA, DALILA ALDANA-ARANDA, and ERICK R. BAQUEIRO-CÁRDENAS** 93

Daedalochila sp. nov. from northwest Arkansas, U.S.A., the anatomy of the *Polygyra plicata* group, and the validity of the genus *Millerelix* Pratt, 1981 (Gastropoda: Pulmonata: Polygyridae). **BRIAN F. COLES and GERALD E. WALSH** 99

Research Note 113

Book Review 117

Meeting Announcement 119

Financial Report 121

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Amer. Malac. Bull. **21**: 1-10

Evaluation of a recirculating pond system for rearing juvenile freshwater mussels at White Sulphur Springs National Fish Hatchery, West Virginia, U.S.A.

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Abstract: A recirculating double-pond system at White Sulphur Springs National Fish Hatchery in West Virginia, U.S.A., was evaluated for suitability for culturing juvenile freshwater mussels. Newly metamorphosed juveniles of *Villosa iris* and *Lampsilis fasciola* were placed in the system, and their growth and survival were evaluated for 94 days. Throughout the study, parameters of water quality remained within ranges suitable for mussel survival. Planktonic algal densities in the pond system ranged from 2850 to 6892 cells/ml. Thirty-seven algal taxa were identified, primarily green algae (Chlorophyta), diatoms (Bacillariophyceae), and blue-green algae (Cyanoprokaryota). Over the culture period, juveniles of *L. fasciola* experienced significantly lower ($p < 0.001$) survival (6.3% ± 4.5) than those of *V. iris* (49.8% ± 14.5). The very low survival rate of *L. fasciola* may indicate a failure of the flow-through pond environment to meet its habitat requirements or that variable microhabitat conditions within culture containers existed. Growth did not differ significantly between the species ($p = 0.13$). Survival of *V. iris* and growth of both species were similar to previous trials to culture juvenile mussels. Survival rates as high as 66.4% at 93 days for *V. iris* suggest that juveniles of some riverine species can be successfully cultured in a recirculating pond environment.

Key words: captive culture, conservation hatchery, *Villosa iris*, *Lampsilis fasciola*

Populations of freshwater mussels have declined dramatically in recent decades, with over 70% of North America's mussel species considered to be endangered, threatened, or of special concern (Williams *et al.* 1993). Declines are attributable to many factors, including habitat alteration, competition with exotic species such as the Asian clam (*Corbicula fluminea* Müller, 1774) and zebra mussel (*Dreissena polymorpha* Pallas, 1771), siltation, and degraded water quality. Once mussel populations decline, recolonization rates are slow, even if environmental conditions improve (Ahlstedt 1979). Recovery plans for endangered species of mussels often recommend propagation and reintroduction (National Native Mussel Conservation Committee 1998), as reintroduction can restore species into historical ranges and augment existing populations.

Ponds and raceways of fish hatcheries are potential locations for artificial propagation and grow-out of juvenile mussels (National Native Mussel Conservation Committee 1998). Studies of feeding habits of juvenile mussels suggest that natural environments may provide nutrients, vitamins, or sediment-associated bacteria not provided in laboratory conditions (Gatenby *et al.* 1996). Outdoor ponds and raceways are colonized by aquatic insects, algae, bacteria, protozoans, and other microorganisms and also accumulate organic detritus. Thus, these facilities simulate the aquatic community that would occur in a local river, and some of the benefits of a natural environment may be achieved. Additionally, the use of outdoor facilities may be less labor-intensive than the maintenance of indoor laboratory conditions.

Habitat requirements of the juvenile phases of most species of mussels are not well understood (Kat 1982, Gordon and Layzer 1989) and this lack of information provides challenges for propagating mussels in a controlled environment. For a facility to be useful for propagation, it should provide suitable habitat and an adequate food supply for multiple species.

The purpose of this study was to evaluate the growth and survival of juveniles of two species of mussels of the unionid subfamily Lampsilinae, released into a recirculating double-pond system located at White Sulphur Springs National Fish Hatchery in West Virginia, U.S.A.

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METHODS

Establishing appropriate conditions for growing juvenile mussels

Within the recirculating double-pond system (Fig. 1), juvenile mussels were held in the mussel-rearing pond, while the second pond was maintained to foster the growth of algae. The mussel-holding pond was modified to create a flow-through raceway environment for the grow-out of juvenile mussels. Both ponds were approximately 11 m wide by 84 m long and fitted with commercial pond liners. Six elevated cinderblock troughs were constructed in the mussel pond to confine the containers holding juvenile mussels. Six rectangular plastic containers (20 cm x 40 cm x 15 cm deep) were placed randomly in 2 rows in the upper third of trough 2 at a depth of approximately 40 cm. To supplement the nutrient base and natural algal community in the mussel pond, 50 burlap bags containing leaf detritus (average weight of 6.8 kg/bag) were added in September and October of 1999 and 2000, prior to the initiation of this study.

Both ponds were supplied initially with 12°C water from 2 springs (an upper and lower spring) and a well located on the hatchery premises. Water was recirculated between the ponds through 5 cm waterline and 5 cm PVC pipes using two 0.5 horsepower submersible pumps (Peabody Barnes model 35E54) that pumped approximately 454 L/min. Supplemental water was added as needed to maintain water levels and to lower water temperature when it exceeded 25°C. Water recirculation allowed a retention time adequate for solar radiation to warm the water to greater than 15°C, the temperature necessary to support growth in juvenile mussels (Hanlon 2000). Water entering the mussel pond was directed through a PVC manifold with four outflow pipes for the six elevated troughs (only one trough contained mussels for this experiment). A paddle-wheel aerator (Aqua and Co., Model Eolo 2) was placed in the upper end of the mussel pond to increase dissolved oxygen and water velocity. Stop boards at the outlet structure of the algal pond maintained a depth of 1.4 m, resulting in a depth of 0.7 m at the upper end and a volume of approximately 819,000 L. Stop boards in the mussel pond maintained a depth of approximately 1.1 m, resulting in a depth of 0.4 m at the upper end and a volume of approximately 545,000 L. Based on the pumping rate and total water volume, the calculated time to fully exchange water between the two ponds was approximately 24 h.

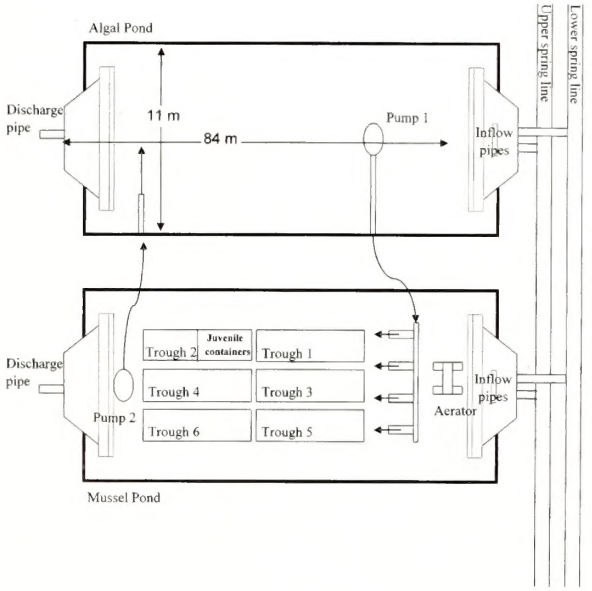

Figure 1. Schematic drawing of the recirculating double-pond system for culturing juvenile mussels at White Sulphur Springs National Fish Hatchery, West Virginia.

Monitoring of habitat parameters and water quality

Water velocity was measured with a pygmy flow meter on cross-sections of the raised troughs holding the containers for juvenile mussels. Temperature was recorded continuously with a Hobo™ (Onset Corporation) temperature logger. Hardness, alkalinity, pH, dissolved oxygen, orthophosphate, nitrate, nitrite, and total ammonia were measured bi-weekly. Dissolved oxygen was measured with a YSI meter (model 85), and pH was measured with an Orion meter (model 290A). Hardness and alkalinity were measured with Hach™ test kits. Orthophosphate, nitrate, nitrite, ammonia, and ammonium were analyzed according to standard methods (Eaton *et al.* 1995). Total ammonia was measured with an Orion ion-selective probe and an Orion meter (model 290A). The proportion of un-ionized ammonia (NH_3-N) was determined using the formulas of Emerson *et al.* (1975) with the measured parameters of temperature and pH. Nitrite and nitrate concentrations were measured by spectrophotometric analysis using the cadmium reduction and sulfanilamide method. Orthophosphate was measured with spectrophotometric analysis using the ascorbic acid-molybdate method.

Algal production and monitoring

The dominant genera and density of algae in the water column of the two ponds were monitored every 2 weeks. Water samples were collected in Nalgene bottles (1 L) at 20 cm depth and immediately preserved with 10 ml of acid Lugol's fixative (Saraceni and Ruggio 1969). According to the Utermohl technique, a 100 ml settling chamber was used to concentrate the algae in the water sample. Algae settled onto a slide for 48 hr, then were identified and enumerated using an inverted microscope at 200X magnification (Vollenweider 1969). Genera were identified according to a di-

chotomous key (Prescott 1978) and counted by transect. Cell densities were then calculated. All observed genera were classified as potentially ingestible or unlikely to be ingested by juvenile mussels. Ingestibility was evaluated based on previous studies of gut contents of juvenile mussels (Yeager *et al.* 1994, Gatenby *et al.* 1996) and size, shape, cellular make-up, and growth form (Beck and Neves 2003). The ingestible proportion of algal cells in each sample was then calculated. Filamentous algae were identified with a standard binocular microscope at 400× magnification.

Preliminary analyses of total inorganic nitrogen and soluble reactive phosphate-phosphorus of the water showed a 4:1 ratio of N:P. To increase this ratio to 15:1, the level most suitable for unicellular green algae and diatoms (Redfield 1958, Hillebrand and Sommer 1999), a fertilization regime of 1.4 kg ammonium nitrate was applied once per retention time of approximately 1 month. Additional fertilization occurred according to observed algal densities and measured nutrient levels.

Comparison of growth and survival of juveniles

Collection of host fish and production of juvenile mussels were accomplished by standard techniques (Zale and Neves 1982). Glochidia were collected from ten gravid females of *Vilosa iris* (Lea, 1829) and five gravid *Lampsilis fasciola* (Rafinesque, 1820). Largemouth bass (*Micropterus salmoides* Lacepédè, 1802) and smallmouth bass (*Micropterus dolomieu* Lacepédè, 1802) served as hosts for *L. fasciola* and rockbass (*Ambloplites rupestris*, Rafinesque, 1817) served as the host for *V. iris*. A total of 1500 juveniles of *V. iris* (7-10 days old) and 1500 juveniles of *L. fasciola* (1-5 days old) were collected, and initial lengths of 10 juveniles of each species were measured. Juvenile mussels were then transferred to the hatchery and acclimated by gradually replacing their water with hatchery water in 20% increments over 8 hr.

Juvenile mussels were transferred to six plastic containers (500 mussels per container; 3 containers per species) with substrate (depth approximately 0.5 cm) consisting of a 1:1 mixture of quarried limestone sand sieved to a particle size between 1000-3000 μm and river bottom sediment from Little River, Tazewell County, Virginia (sieved to particle size 800-1500 μm), to a depth of 0.5 mm. Mixed particle sizes were used to increase the availability of interstitial spaces for movement and feeding of the juveniles.

At 2-week intervals, juvenile mussels were sieved from the substrate of 3 randomly selected containers to assess their growth and survival. A rotational sampling schedule was employed to ensure that each container was sampled at least three times over the study period but was not sampled consecutively. This was accomplished with the exception of one container that was sampled consecutively early in the study. All live juveniles in the sampled containers were counted to estimate survival rates, and a subsample of 10 individuals was measured using an ocular micrometer to record growth. While mussels were sampled at the same time, the two species were slightly different in their ages at introduction, thus there was an age difference in the mussels at the time of sampling.

Growth and survival were compared between species. Growth was evaluated with a general linear model multivariate analysis of variance test, using a paired t-test on the mean lengths at each sampling time (SAS 1990). Because growth was measured from a subsample of juveniles, growth data were considered to be independent. Survival was compared as binomial proportions (based on the proportion surviving) and the data were arc-sine transformed to achieve a normal distribution (Sokal and Rohlf 1995). At each sampling event, survival for a given container was not independent of previous survival rates. Therefore, survival was compared with a mixed procedure multivariate analysis of variance test, intended to model and account for the covariance structure of the data (SAS 1990).

RESULTS

Parameters of water quality

The distributed flow across the pond was approximated at 1.4 ml min^{-1} cm^{-2}, assuming uniform flow throughout the cross-section of the pond. Specific velocity measurements were 4.8 cm/s immediately below the PVC manifold outlet into trough 1 and 4.6 cm/s at the midpoint of trough 1. Velocities at the bottom of trough 1 and at the top of trough 2 were too low for detection with the flow meter.

Mean daily temperatures observed in the mussel pond for time intervals between sampling events ranged from 21.0°C to 25.6°C. The minimum recorded temperature was 16.8°C, and the maximum was 31.3°C. In the mussel pond, hardness was 305.7 mg/l (largely due to high sulfate levels), alkalinity was 71.4 mg/l, dissolved oxygen averaged 8.9 mg/l, and pH was 8.5 (Table 1). Total ammonia in the mussel pond ranged from 0.05 to 0.21 mg (NH_3-N + NH_4-N)/l, with a mean of 0.09 (± 0.05) mg (NH_3-N + NH_4-N)/l. Calculated levels of un-ionized ammonia ranged from 0.003 to 0.06 mg NH_3-N/l. Nitrate concentrations in the mussel pond ranged from 0.005 to 0.14 mg NO_3-N/l, with a mean of 0.04 (± 0.04) mg NO_3-N/l. Nitrite levels were detectable on only two sampling dates, at concentrations of 0.001 and 0.03 mg NO_2-N/l. Orthophosphate levels in the mussel pond were low throughout the study period; 5 of 8 samples had undetectable levels, and the maximum level was 0.05 mg PO_4-P/l.

Algal production

We identified and counted 36 taxa of algae in water samples from the mussel pond, 5 of which also appeared in

Table 1. Values of chemical parameters from water samples from the mussel pond and algal pond 7 June-12 October 2001.

Parameter	Pond	Mean ± SD	Range	N
Hardness (mg/l)	mussel	305.7 ± 79.8	200-420	7
Alkalinity (mg/l)	mussel	71.4 ± 15.7	60-100	7
Dissolved Oyxgen (mg/l)	mussel	8.92 ± 0.52	8.44-10.09	7
pH	mussel	8.48 ± 0.25	8.18-8.65	7
Total Ammonia	mussel	0.087 ± 0.054	0.052-0.21	8
(mg [NH_3-N+NH_4-N]/l)	algae	0.058 ± 0.018	0.033-0.089	6
Unionized Ammonia	mussel	0.015 ± 0.020	0.002-0.060	7
(mg NH_3-N/l)				
Nitrate (mg NO_3-N/l)	mussel	0.035 ± 0.043	0.005-0.14	8
	algae	0.025 ± 0.015	0.010-0.043	5
Nitrite (mg NO_2-N/l)	mussel	0.016 ± 0.021	0.001-0.03	8
Orthophosphate	mussel	0.031	n/a**	8
(mg PO_4-P/l)	algae	0.033 ± 0.016	0.014-0.052	7

** SD and range n/a, orthophosphate was detectable only in one sample.

samples from the algal pond (Table 2). The observed genera included unicellular, colonial, and filamentous taxa. Six groups of algae were represented; 17 genera of green algae, 12 diatoms, 5 blue-green algae, 1 golden alga, 1 cryptophyte, and 1 euglenoid. The top 10 taxa most frequently observed among all samples were *Oscillatoria, Cocconeis, Diatoma, Cromulina, Chroomonas, Chlamydomonas, Chlorella, Chlorococcum, Protoderma,* and *Scenedesmus.* Green algae comprised from 58.4 to 79.6% of the cells observed in the samples (Table 3). Diatoms ranged from 4.3 to 19.8% of the cells observed, and blue-greens ranged from 2.7 to 18.4%. Algal diversity was not significantly different between the two ponds ($p = 0.38$).

Densities of algal cells ranged from 2850 cells/ml to 6892 cells/ml, with a mean of 4262 cells/ml in the mussel pond and 3681 cells/ml in the algal pond. Mean cell density did not differ significantly between the two environments (2-sample t-test assuming unequal variance, $p = 0.38$). Proportions of algal cells that were potentially ingestible ranged from 65.4-88.8% (Table 3). Macroscopic filamentous algae heavily colonized the algal pond in early July 2001 but were less abundant in the mussel pond. The dominant filamentous genera were *Sphaeroplea* and *Cladophora.*

Comparison of growth and survival of *Villosa iris* and *Lampsilis fasciola*

Sampling occurred on the same days for evaluation of growth and survival, however, because of the earlier culture of *Lampsilis fasciola*, they were 2-3 days younger than *Villosa iris* on the sampling dates. The slight age difference was assumed not to have an influence on the results. At the end of the experiment, mean survival for *V. iris* (mean age 93 days) was 49.8% (±14.5) and mean survival for *L. fasciola* (mean age 86 days) was 6.3% (±4.5). On all five sampling events, mean survival was higher for *V. iris* than for *L. fasciola* (Fig. 2). In contrast to *V. iris*, survival declined sharply for *L. fasciola* over the first two sampling dates and then stabilized. Over the culture period there was a significant difference ($p < 0.01$) in survival between species. Significant differences ($p = 0.01$) in survival between different containers of the same species indicated that conditions within individual containers may have affected survival.

At the end of the experiment, shell lengths of surviving *Villosa iris* ranged from 0.75 to 3.21 mm, with a mean length of 1.81 (±0.67) mm; shell lengths of *Lampsilis fasciola* ranged from 0.75 to 3.59 mm with a mean length of 1.78 (±0.78) mm (Fig. 3). The multivariate ANOVA did not show a statistical difference in growth between the two species ($p = 0.13$).

DISCUSSION

Comparison of growth and survival of *Villosa iris* and *Lampsilis fasciola*

The two species exhibited very different survival rates. Overall, survival of *Villosa iris* was good, while survival of *Lampsilis fasciola* was poor. A number of factors may have contributed to the low survival of *L. fasciola.* Our experiences in culturing mussels have shown that there is high variability in survival rates among broods of mussels. Factors ranging from the maturity of glochidia at the time of extrac-

Table 2. Genera of algae recorded in water samples taken from the mussel and algal ponds, 5 July-15 September 2001. Growth form (C = colonial, F = filamentous, Ps = pseudofilament, U = unicellular), habitat occupied (B = benthic, P = planktonic), and pond found in (algal pond, mussel pond, or both ponds) presented in parentheses. Taxonomy based on Prescott (1978).

Algae (growth form/habitat)	Algae (growth form/habitat)
Blue Green Algae (Cyanophyta)	Golden Algae (Chrysophyceae)
Aphanocapsa (C/P; algal)	*Cromulina** (U/P; both)
Chroococcus (C/B; both)	Cryptophytes (Cryptophyta)
Lyngbya (F/B or P; both)	*Chroomonas** (U/P; both)
*Oscillatoria** (F/B or P; both)	Euglenoids (Euglenophyta)
Spirulina (F/P; mussel)	*Euglena* (U/P; both)
Green Algae (Chlorophyta)	Diatoms (Bacillariophyceae)
*Chlamydomonas** (U/P; both)	*Cocconeis** (U/B; both)
*Chlorella** (U/P; both)	*Cyclotella* (U/P; both)
*Chlorococcum** (U/B or P; both)	*Cymbella* (U/B; mussel)
Chodatella (U/P; mussel)	*Diatoma** (U/B; both)
Closterium (U/B or P; mussel)	*Fragilaria* (C/P/B; both)
Koliella (U/P; algal)	*Meridion* (U/B; both)
Monocilia (F/B; algal)	*Navicula* (U/B; both)
Mougeotia (F/B; both)	*Pinnularia* (U/B; both)
Oedogonium (F/B; mussel)	*Synedra* (U/P; both)
Oocystis (U/P; both)	*Tabellaria* (Ps/P; mussel)
Pediastrum (U/P; both)	*Gomphonema* (U/B; mussel)
*Protoderma** (C/B; both)	
Schroederia (C/P; both)	
Selenastrum (C/P; both)	
*Scenedesmus** (C/P; both)	
Sphaeroplea (F/B; mussel)	
Ulothrix (F/B; mussel)	

* 10 most frequently occurring genera.

tion to the conditions during the infestation period can affect the robustness of a cohort of juveniles. Different rates of survival between the species may indicate a failure of the flow-through pond environment to meet the habitat requirements of *L. fasciola* or that variable conditions within culture containers were responsible for the lower survival of *L. fasciola*.

Habitat conditions

Flow is important to juvenile mussels to flush wastes and to bring food to the sediment-water interface (Yeager *et al.* 1994, Hanlon 2000). The distributed flow across the pond of 1.4 ml $min^{-1}cm^{-2}$ is lower than the 5 ml $min^{-1}cm^{-2}$ cited as appropriate for culturing other juvenile bivalves and found suitable by Steg (1998). Juvenile mussels often inhabit different microhabitats than adults and are often found in depositional areas with slow velocities, such as behind boulders where flow rates may be lower (Neves and Widlak 1987). Nevertheless, the low flow rate appears to be suitable for *V. iris*.

Temperatures in the algal pond were above the minimum threshold of 15°C required for the growth of juvenile mussels (Hanlon 2000) and were also suitable for the production of algae. Water temperatures of around 20°C are generally recommended as most suitable for the growth and survival of juvenile mussels (Steg 1998, Beaty 1999). In this study, the mean temperature of 22.6°C was close to this reported value. Maximum daily temperatures exceeded 25°C on 43 days; however, the highest mean and daily maximum temperatures occurred during late July and the first half of August. Neither declines in survival nor reduction in relative growth rates were correlated with these elevated temperatures, so it is unlikely that these higher temperatures had deleterious effects.

Water quality

The parameters of water quality recorded throughout the study were within the ranges suitable for juvenile mussels (O'Beirn *et al.* 1998). Levels of orthophosphate were low but similar to levels (mean 0.15 mg/l) recorded in habitats that support mussel populations (Strayer 1999). In spite of our increased frequency of nutrient addition, nitrate levels remained less than 0.05 mg NO_3-N /l in all water samples.

Mean total ammonia in the mussel pond was 0.07 (±0.02) mg (NH_3-N + NH_4-N)/l, which is higher than environmental levels measured in mussel habitats in the Clinch, Powell, and Holston river systems, where total ammonia concentrations typically are less than the 0.04 mg (NH_3-N + NH_4-N)/l detectable limit (Virginia Department of Environmental Quality 2001). Ammonia's toxicity to aquatic organisms is well documented, attributed largely to the fraction of ammonia occurring in the un-ionized form (U.S. E.P.A. 1984). Levels of un-ionized ammonia reported to be acutely toxic to young bivalves range from 0.10 to 2.0 mg NH_3-N /l (Goudreau *et al.* 1993, Scheller 1997, Summers 1998, Mummert *et al.* 2003), higher than those recorded for the mussel pond.

Algae as a food source

The growth of juvenile mussels in this study compared favorably to those of other studies of the culture of freshwater mussels (Table 4). When an adequate food source or substrate for rearing juveniles of *V. iris* is not provided in laboratory culture, juveniles cease to grow beyond a mean length of approximately 0.45 mm (Gatenby 1994). Juveniles of both species exceeded this length by age 21 days, indicating that their nutritional and substrate needs were met. Additionally, juvenile mussels examined in this study exhibited green and brown coloration of their guts, indicating they

Table 3. Summary of the characteristics of the algal communities in the mussel pond (MP) and algal pond (AP) based on major type, diversity, % ingestible by juvenile mussels (as based on Yeager *et al.* 1994 and Gatenby *et al.* 1996), and density on each sample date, 5 July-14 September 2001.

	7/5/01		7/19/01	8/1/01		8/16/01		8/29/01		9/14/01	
Category (%)	MP	AP	MP	MP	AP	MP	AP	MP	AP	MP	AP
Diatoms	16.5	10.3	12.8	14.7	4.3	12.3	19.8	9.9	7.3	7.6	11.5
Green Algae	71.5	75.1	77.2	64.7	78.1	72.2	58.4	72.2	79.6	73.2	68.2
Bluegreen Algae	3.2	13.0	2.7	18.4	15.8	7.9	10.8	16.9	7.6	15.4	14.8
Other	8.2	1.6	7.3	2.2	1.8	7.6	11.0	1.1	5.5	3.8	5.5
Diversity											
(No. of genera)	24	21	17	20	16	20	18	20	20	18	18
% Ingestible	88.4	81.7	84.9	72.9	65.4	82.2	85.3	80.8	88.8	81.7	81.1
Density (cells/ml)	6892	4382	3854	4339	4305	3284	3710	3605	2850	3599	3157

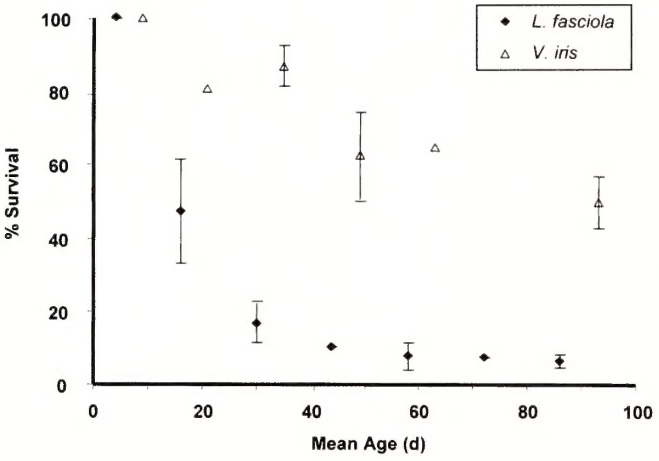

Figure 2. Mean survival (±1 SD) of juveniles of *Villosa iris* and *Lampsilis fasciola* reared in a recirculating double-pond system over a 94 day study period.

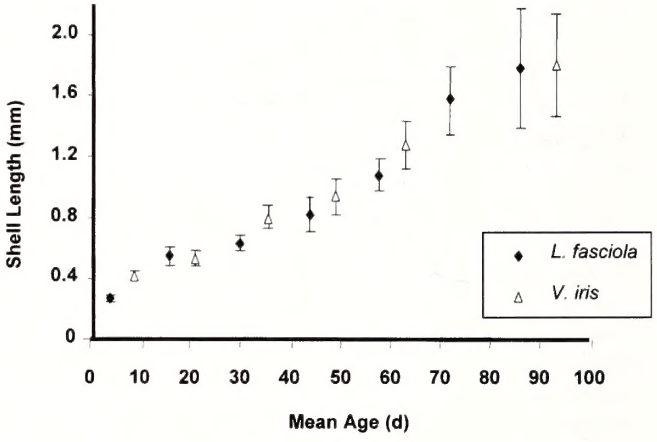

Figure 3. Mean shell lengths (±1 SD) of juveniles of *Villosa iris* and *Lampsilis fasciola* reared in a recirculating double-pond system over a 94 day study period.

had ingested chlorophyll-containing algae and other food particles.

Previous studies have shown that phytoplankton densities 2-30 times above those observed in our study are optimal for filter-feeding adult bivalves. Maximum ingestion efficiency occurs at algal densities between 11,000-15,000 cells/ml for adults of the freshwater mussel *Elliptio complanata* (Lightfoot, 1786) (Paterson 1984) and at densities from 75,000-100,000 cells/ml for some marine bivalves (Rajesh *et al.* 2001). However, whereas adult bivalves filter phytoplankton from the water column, juvenile mussels acquire particulate nutrients from the sediment by pedal feeding and by filtering interstitial water (Yeager *et al.* 1994). Thus, high concentrations of planktonic algae may not be required to meet their nutritional requirements. Additionally, recent investigations have revealed that bivalves feed on a full array of naturally occurring particles including bacteria, protozoans, and phytoplankton (Baldwin and Newell 1991, Nichols and Garling 2000). Nichols and Garling (2000) found that algal-derived vitamins and lipids were accumulated by adult unionids, but that bacteria were the main source of stored carbons, with bacterially-derived compounds, notably vitamin B_{12}, playing a key dietary role. In the mussel pond, other biota and detritus may have provided supplemental nutrition to augment the phytoplankton.

Estimates of concentrations of phytoplankton that bivalves encounter in other freshwater natural systems are extremely variable, with densities of less than 5,000 cells/ml widely reported (Stoyneva and Dragnov 1991, Stevenson and White 1995). In some cases densities are less than 1,000

Table 4. Summary of the most successful growth and survival rates reported in studies of the culture of juvenile freshwater mussels *Villosa iris* and *Lampsilis fasciola.*

Species	Age at end of study (d)	Mean % Survival	Mean Length (mm)	Type of Culture System	Source
V. iris	91-94	49.8	1.81	Spring/well-fed outdoor flow-through pond	This study
V. iris	91-94	32.9	1.78	Spring/well-fed outdoor flow-through pond	This study
V. iris	140	30.0	1.80	Indoor downweller	Gatenby *et al.* 1997
V. iris	115	27.5	2.10	River-fed artificial stream	Beaty 1999
V. iris	154	26.8	2.7	Indoor recirculating aquaculture system	O'Beirn *et al.* 1998
L. fasciola	83-88	6.3	1.78	Spring/well-fed outdoor flow-through pond	This study
L. fasciola	90	82.0	2.23	River-fed outdoor flow-through raceway	Hanlon 2000
L. fasciola	166	74.6	2.28	River-fed outdoor flow-through raceway	Hanlon 2000
L. fasciola	122	50	1.76	River-fed outdoor flow-through raceway	Hanlon 2000
L. fasciola	105	47.3	2.13	Indoor recirculating aquaculture system	Steg 1998
L. fasciola	200	41.2	1.59	River-fed outdoor flow-through raceway	Hanlon 2000

cells/ml (Wilhm *et al.* 1977, Boltovskoy *et al.* 1995). Additionally, phytoplankton densities reported within a given river system usually vary seasonally (Gale and Lowe 1971, Davis *et al.* 1997, Wehr and Thorp 1997, Patterson *et al.* 1999). For example, estimates of densities measured monthly from January through December from the Mississippi River ranged from 5,000-55,000 cells/ml (Gale and Lowe 1971). Thus mussels in natural habitats survive on variable densities of algae and particulates.

The genera of algae recorded in our study ponds were of high quality as sources of food. Polyunsaturated fatty acids (PUFA) promote growth in juvenile freshwater mussels (Gatenby *et al.* 1996) and diatoms, generally rich in PUFA (Pohl and Zurheide 1979), were prevalent in our samples. Twelve genera of diatoms contributed a mean of 12.3% of the cells observed in the samples from the mussel pond, and 10.6% of the cells in the samples from the algal pond. Two diatom genera (*Diatoma* and *Cocconeis*) were among the top 10 dominant genera in all samples. Members of the Cryptophyceae also produce some PUFA and the cryptophyte genus *Chroomonas* was among the top 10 most frequently observed taxa. Other nutrients of potential importance to the nutrition of juvenile mussels include sterols and steroids, which occur in all the algal groups observed except the blue-green algae (Pohl and Zurheide 1979). Carotenoids, which influence the growth of marine mussels, can occur in all of the algae, including the blue-green genera (Campbell 1969, Gatenby 1994). Because mixed algal diets are prefered to single species diets for rearing marine bivalves (Epifanio 1979, Romberger and Epifanio 1981), because greater algal diversity has been correlated with increased growth (Shpigel *et al.* 1993), and because of the good growth of *V. iris*, the density of algae in this study likely was adequate for nutrient needs of juvenile mussels.

Juvenile mussels are known to ingest a number of the algal genera observed in the mussel pond. *Clamydomonas* and *Scenedesmus*, two of the dominant genera, have been suggested for freshwater bivalve culture (Foe and Knight 1986, Lauritsen 1986). In laboratory feeding studies, *Chlamydomonas reinhardtii* (Dangeard, 1888) was ingested and found partially digested in the gut of juvenile mussels (Gatenby *et al.* 1996). *Chlorella* constituted the dominant genus in all samples (38.3-56.8%) and *Chlorella vulgaris* has been found previously in gut contents of juvenile *Villosa iris* (Yeager *et al.* 1994, Gatenby *et al.* 1996). A number of the genera observed in the recirculating double-pond system (notably *Chlorella, Chlorococcum, Cyclotella, Diatoma, Navicula, Pediastrum,* and *Scenedesmus*) have also been found

in the guts of adult freshwater mussels (Parker *et al.* 1998, Nichols and Garling 2000).

Suitability of the double-pond system for juvenile mussels

Overall, the double-pond system produced a promising environment for culture of juvenile freshwater mussels. Growth in terms of shell lengths was similar to those of previous efforts to grow juvenile mussels under captive conditions (Table 4). Two experiments at the Buller Hatchery in Marion, Virginia, U.S.A. (Hanlon 2000), resulted in mean lengths less than those observed in this study, and those lengths were obtained for older juveniles. The largest individuals in this study (3.59 mm) were nearly as large as the largest juveniles (4.13 mm) observed by Hanlon (2000). Steg (1998) observed a range of sizes (1.4-4.5 mm at 105 days) in a laboratory culture environment, similar to the range in our study. Failure to reach a length of about 0.5 mm in 6 weeks (42 days) has been cited as a benchmark below which juvenile mussels are incapable of over-wintering (Hanlon 2000, Beck and Neves 2003). Even the smaller juveniles in our study exceeded this benchmark. Among juveniles of *Villosa iris* at 34 days, the smallest measured shell length was 0.50 mm, with mean shell lengths ranging from 0.77 mm to 0.80 mm among the containers. Among *Lampsilis fasciola* at age 44 days, the smallest measured shell length was 0.50 mm and mean shell length was 0.80 mm. Thus, individuals in this study achieved lengths adequate for survival upon release to the wild, and larger individuals grew to lengths similar to the largest sizes documented for these species in previous culture studies.

Survival rates of juvenile *Villosa iris* in this study also compare favorably to other survival rates (Table 4). With survival as high as 66.4% at 93 days for *V. iris*, our results suggest that juveniles of at least some riverine species can be successfully cultured in a recirculating pond environment. Further study of the effects of flow rate on survival and growth of juveniles is needed, as well as research to elucidate the roles and importance of microhabitat factors that affect variability in survival among containers.

The cause for low survival rates of *Lampsilis fasciola* is unknown. Variability is known to occur among broods and the use of multiple females to collect glochidia should have addressed this issue. Shell lengths were similar between the two species, thus the surviving *L. fasciola* appeared to have the nutritional requirements needed. Further investigation is required before concluding that *L. fasciola* are not able to be cultured in this type of system.

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LITERATURE CITED

Ahlstedt, S. A. 1979. Recent mollusc transplants in the North Fork Holston River in southwest Virginia. *Bulletin of the American Malacological Union* [for 1979]: 21-23.

Baldwin, B. S. and R. I. E. Newell. 1991. Omnivorous feeding by planktotrophic larvae of the eastern oyster *Crassostrea virginica*. *Marine Ecology Progress Series* **78**: 285-301.

Beaty, B. B. 1999. *Development of Juvenile Culture Techniques and Testing of Potential Biomarkers of Environmental Stress in Freshwater Mussels (Bivalvia: Unionidae)*. Ph.D. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Beck, K. M. and R. J. Neves. 2003. An evaluation of selective feeding by three age-groups of the rainbow mussel *Villosa iris*. *North American Journal of Aquaculture* **65**: 203-209.

Boltovskoy, D., I. Izaguirre, and N. Correa. 1995. Feeding selectivity of *Corbicula fluminea* (Bivalvia) on natural phytoplankton. *Hydrobiologia* **312**: 171-182.

Campbell, S. A. 1969. Seasonal cycles in the carotenoid content in *Mytilus edulis*. *Marine Biology* **4**: 227-232.

Davis, L. N., K. A. Phillips, and H. G. Marshall. 1997. Seasonal abundance of autotrophic picoplankton in the Pagan River, a nutrient enriched subestuary of the James River, Virginia. *Virginia Journal of Science* **48**: 212-217.

Eaton, A., L. Clesceri, and A. Greenberg. 1995. *Standard Methods for the Examination of Water and Wastewater*, 19th Ed. The American Public Health Association, Washington, D.C.

Emerson, K., R. C. Russo, R. E. Lund, and R. V. Thurston. 1975. Aqueous ammonia equilibrium calculations: Effects of pH and temperature. *Journal of the Fisheries Research Board of Canada* **32**: 2379-2383.

Epifanio, C. E. 1979. Growth in bivalve molluscs: Nutritional effects of two or more species of algae in diets fed to the American oyster *Crassostrea virginica* (Gmelin) and the hard clam *Mercenaria mercenaria* (L.). *Aquaculture* **18**: 1-12.

Foe, C. and A. Knight. 1986. A thermal energy budget for *Corbicula fluminea*. *American Malacological Bulletin, Special Edition* **2**: 143-150.

Gale, W. F. and R. L. Lowe. 1971. Phytoplankton ingestion by the fingernail clam, *Sphaerium transversum* (Say), in pool 19, Mississippi River. *Ecology* **52**: 507-513.

Gatenby, C. M. 1994. *Development of a Diet for Rearing Juvenile Freshwater Mussels.* M.S. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Gatenby, C. M., R. J. Neves, and B. C. Parker. 1996. Influence of sediment and algal food on cultured juvenile freshwater mussels. *Journal of the North American Benthological Society* **12**: 148-156.

Gatenby, C. M., R. J. Neves, and B. C. Parker. 1997. Growth and survival of juvenile rainbow mussels, *Villosa iris* (Lea, 1829) (Bivalvia: Unionidae), reared on algal diets and sediment. *American Malacological Bulletin* **14**: 57-66.

Gordon, M. E. and J. B. Layzer. 1989. Mussels (Bivalvia: Unionidae) of the Cumberland River: A review of life histories and ecological relationships. *U.S. Fish and Wildlife Service, Biological Report* **89**: 1-99.

Goudreau, S. E., R. J. Neves, and R. J. Sheehan. 1993. Effects of wastewater treatment plant effluents on mollusks in the upper Clinch River, Virginia, USA. *Hydrobiologia* **252**: 211-230.

Hanlon, S. D. 2000. *Release of Juveniles into a Fish Hatchery Raceway: A Comparison of Techniques.* M.S. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Hillebrand, H. and U. Sommer. 1999. The nutrient stoichiometry of benthic microalgal growth: Redfield proportions are optimal. *Limnology and Oceanography* **44**: 440-446.

Kat, P. W. 1982. Effects of population density and substratum type on growth and migration of *Elliptio complanata* (Bivalvia: Unionidae). *Malacological Review* **15**: 119-127.

Lauritsen, D. D. 1986. Filter-feeding in *Corbicula fluminea* and its effect on seston removal. *Journal of the North American Benthological Society* **5**: 165-172.

Mummert, A. K., R. J. Neves, T. J. Newcomb, and D. S. Cherry. 2003. Sensitivity of juvenile freshwater mussels (*Lampsilis fasciola, Villosa iris*) to total and un-ionized ammonia. *Environmental Toxicology and Chemistry* **22**: 2545-2553.

National Native Mussel Conservation Committee. 1998. National strategy for the conservation of native freshwater mussels. *Journal of Shellfish Research* **17**: 1419-1428.

Neves, R. J. and J. C. Widlak. 1987. Habitat ecology of juvenile freshwater mussels (Bivalvia: Unionidae) in a headwater stream in Virginia. *American Malacological Bulletin* **5**: 1-7.

Nichols, S. J. and D. Garling. 2000. Food-web dynamics and trophic-level interactions in a multispecies community of freshwater unionids. *Canadian Journal of Zoology* **78**: 871-882.

O'Beirn, F. X., R. J. Neves, and M. B. Steg. 1998. Survival and growth of juvenile freshwater mussels (Unionidae) in a recirculating aquaculture system. *American Malacological Bulletin* **14**: 165-171.

Parker, B. C., M. A. Patterson, and R. J. Neves. 1998. Feeding interactions between native freshwater mussels (Bivalvia: Unionidae) and zebra mussels (*Dreissena polymorpha*) in the Ohio River. *American Malacological Bulletin* **14**: 173-179.

Paterson, C. G. 1984. A technique for determining apparent selective filtration in the freshwater bivalve *Elliptio complanata* (Lightfoot) in an old reservoir in New Brunswick, Canada. *Freshwater Invertebrate Biology* **4**: 201-207.

Patterson, M. A., B. C. Parker, and R. J. Neves. 1999. Glycogen concentration in the mantle tissue of freshwater mussels (Bivalvia: Unionidae) during starvation and controlled feeding. *American Malacological Bulletin* **15**: 47-50.

Pohl, P. and F. Zurheide. 1979. Fatty acids and lipids of marine algae and the control of their biosynthesis by environmental factors. *In*: H. A. Hoppe, T. Levring, and Y. Tanaka, eds., *Marine Algae in Pharmaceutical Science.* Walter de Gruyter, New York. Pp. 473-523.

Prescott, G. W. 1978. *How to Know the Freshwater Algae*, 3rd Ed. William C. Brown/McGraw Hill Co., Dubuque, Iowa.

Rajesh, K. V., K. S. Mohamed, and V. Kripa. 2001. Influence of algal cell concentration, salinity and body size on the filtration and ingestion rates of cultivable Indian bivalve. *Indian Journal of Marine Sciences* **30**: 87-92.

Redfield, A. C. 1958. The biological control of the chemical factors in the environment. *American Scientist* **46**: 205-221.

Romberger, H. P. and C. E. Epifanio. 1981. Comparative effects of diets consisting of one or two algal species upon assimilation efficiencies and growth of juvenile oyster, *Crassostrea virginica* (Gmelin). *Aquaculture* **25**: 77-87.

Saraceni, C. and D. Ruggio. 1969. Techniques for sampling water and phytoplankton. *In*: R. A. Vollenweider, ed., *A Manual for Measuring Primary Production in Aquatic Environments.* IBP Handbook No. 12. Blackwell Scientific Publications, Oxford. Pp. 5-7.

SAS. 1990. *SAS/STAT User's Guide*, 4th Ed. SAS Institute, Inc., Cary, North Carolina.

Scheller, J. L. 1997. *The Effect of Die-offs of Asian Clams* Corbicula fluminea *on Native Freshwater Mussels (Unionidae).* M.S. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Shpigel, M., J. Loo, B. Soohoo, R. Friedman, and H. Gordin. 1993. Use of effluent water from fish-ponds as a food source for the Pacific oyster, *Crassostrea gigas* Thunberg. *Aquaculture and Fisheries Management* **24**: 529-543.

Sokal, R. R. and F. J. Rohlf. 1995. *Biometry*, 3rd Ed. W.H. Freeman and Co., New York.

Steg, M. B. 1998. *Identification of Host Fishes and Experimental Culture of Juveniles for Selected Freshwater Mussel Species in Virginia.* M.S. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Stevenson, R. J. and K. D. White. 1995. A comparison of natural and human determinants of phytoplankton communities in the Kentucky River basin, USA. *Hydrobiologia* **297**: 201-216.

Stoyneva, M. P. and S. J. Dragnov. 1991. Green algae in the phytoplankton of the Danube-species composition, distribution, cell numbers and biomass. *Archiv für Protistenkunde* **139**: 243-260.

Strayer, D. L. 1999. Use of flow refuges by unionid mussels in rivers. *Journal of the North American Benthological Society* **18**: 468-476.

Summers, J. M. 1998. *Response of Artificially and Naturally Transformed Juvenile Mussels,* Utterbackia imbecillis, *to Environmental Contaminants.* M.S. Dissertation, Clemson University, Clemson, South Carolina.

U.S. Environmental Protection Agency [USEPA]. 1984. *Ambient Water Quality Criteria for Ammonia.* EPA-440/5-85-001, U.S. Environmental Protection Agency, Washington, D.C.

Virginia Department of Environmental Quality. 2001. Water quality monitoring homepage. Available at: http://www.deq.state.va.us:4100/webapp/wqm.homepage 22 September 2001.

Vollenweider, R. A. 1969. *A Manual on Methods for Measuring Primary Production in Aquatic Environments.* IBP Handbook No. 12, Blackwell Scientific Publications, Oxford and Edinburgh.

Wehr, J. D. and J. H. Thorp. 1997. Effects of navigation dams, tributaries, and littoral zones on phytoplankton communities in the Ohio River. *Canadian Journal of Fisheries and Aquatic Science* **54**: 378-395.

Wilhm, J., T. Dorris, J. R. Seyfer, and N. McClintock. 1977. Seasonal variation in planktonic populations in the Arkansas River near the confluence of Red Rock Creek. *Southwest Naturalist* **22**: 411-420.

Williams J. D., M. L. Warren, K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. *Fisheries* **18**: 6-22.

Yeager, M. M., D. S. Cherry, and R. J. Neves. 1994. Feeding and burrowing behaviors of juvenile rainbow mussels, *Villosa iris* (Bivalvia: Unionidae). *Journal of the North American Benthological Society* **13**: 217-222.

Zale, A. V. and R. J. Neves. 1982. Fish hosts of four species of lampsiline mussels (Mollusca: Unionidae) in Big Moccasin Creek, Virginia. *Canadian Journal of Zoology* **60**: 2535-2542.

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Disappearance of a population of pygmy octopus following a harmful algal bloom in a northwestern Florida bay, U.S.A.

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Abstract: St. Joseph Bay, Bay County, Florida, U.S.A. is 1 of 4 locations at which the pygmy octopus, *Octopus* cf. *mercatoris*, is known to occur. Octopus densities of 1 per 33 m^2 were measured and values of catch per day ranging from 1-70 animals were consistently reported between 1972 and 1999. From 17 August 1999 through 14 October 1999, St. Joseph Bay experienced a severe and prolonged harmful algal bloom (HAB) with cell counts reaching 1,000,000 cells/L at its peak. Populations of *Octopus* cf. *mercatoris* were devastated during the bloom. Repeated and extensive sampling from September 1999 through February 2004 resulted in the collection of only a single brooding female in February 2003. The molluscs' relatively low fecundity (50-320 eggs/clutch) and benthic early life history reduce the probability of distant re-colonization and slow repopulation by surviving octopus within the Bay. Ongoing changes in land use and loss of critical habitat may further stress the remaining, remnant population, leaving them vulnerable to eradication should another HAB event occur.

Key words: cephalopod, red tide, seagrass bed, *Octopus joubini*, *Octopus* cf. *mercatoris*

Octopus joubini (Robson, 1929) had been the only resident species of pygmy octopus within the Gulf of Mexico (Robson 1929, Pickford 1945, Voss 1956, Roper *et al.* 1984) until Forsythe and Toll (1991) demonstrated that the pygmy octopus population of the Gulf of Mexico is actually comprised of two distinct species—*Octopus joubini* and a second, similar species that is either a synonym of *Octopus mercatoris* (holotype collected in the Virgin Islands) or an undescribed species. Although both species are morphologically similar, egg size, fecundity, hatchling type, and habitat preference differ markedly (Forsythe and Toll 1991). Female individuals of *O.* cf. *mercatoris* deposit 50 to 320 eggs ranging in size from 6.0-8.0 mm and emerging young are benthic. Conversely, females *of O. joubini* deposit 150-3000 eggs ranging from 2.3-2.9 mm in length and hatchlings are planktonic. Furthermore, individuals of *O. joubini* are typically limited to sandy or muddy substrates in depths greater than 10 m, whereas individuals of *O.* cf. *mercatoris* favor shallow seagrass beds less than 5 m deep. Within the Gulf of Mexico, *O.* cf. *mercatoris* is thought to be resident in several shallow Florida bays including Biscayne Bay, Palmetto Key, Pine Island, and St. Joseph Bay (Pickford 1945, Forsythe and Toll 1991). Forsythe and Toll (1991) concluded that previously published accounts of pygmy octopus collected from shallow waters in Biscayne and St. Joseph Bays, and widely cited in the literature as *O. joubini*, were in fact *O.* cf. *mercatoris*. Historic taxonomic ambiguities make it difficult to know if *O. joubini* have ever been collected from St. Joseph Bay, therefore we limit our present discussion to populations of *O.* cf. *mercatoris*.

Adult individuals of *Octopus.* cf. *mercatoris* are reclusive, nocturnal hunters that range short distances from their shelters to feed, but are reluctant to leave their dens during the day (Mather 1982b). The proclivity of individuals of *O.* cf. *mercatoris* for shallow water habitats leave them vulnerable to rapid changes in habitat quality; populations in the Gulf of Mexico strike a precarious balance between access to abundant resources in seagrass environments and potential exposure to severe hyperthermal and hypoxic conditions. Temperature and oxygen levels are highly variable in the shallow seagrass environments in the northeastern Gulf of Mexico. For example, high and low daily water temperatures may differ by as much as 10°C (Fangue and Bennett 2003), with mid-day temperatures above 42°C occasionally reported (Harrington and Harrington 1961). Octopus subjected to physiological stress associated with high water temperatures may be further challenged by hypoxia brought on by reduced oxygen solubility and accelerated biological oxygen demand.

Individuals of *Octopus* cf. *mercatoris* routinely endure seasonal and daily abiotic rhythms inherent to their shallow habitats; however, they may be challenged by unpredictable environmental perturbations of unusually severe intensity or duration. For example, a prolonged harmful algal bloom (HAB) lasting from 17 August 1999 to 14 October 1999 appears to have nearly eliminated the once ubiquitous population of *O.* cf. *mercatoris* in St. Joseph Bay, Bay County, Florida, U.S.A. A harmful algal bloom in Florida is defined by a sudden increase in cell count of the causative organism above normal background levels. In the case of *Karenia*

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brevis (=*Gymnodinium breve*), the causative organism in the 1999 HAB, a bloom is considered to be harmful when cell counts exceed 10,000 cells/L (Fish and Wildlife Research Institute 2004b). Previous investigations into the effects of catastrophic HAB events have focused almost exclusively on important game or forage fishes (Anderson 1994, Fish and Wildlife Research Institute 2004a) with little known about possible harmful effects of HABs on endemic octopus populations. The objectives of our study were to describe historical densities of *O.* cf. *mercatoris* in St. Joseph Bay from literature accounts and personal observations, report post-HAB densities based on collections made since August 1999, and assess the re-colonization of this species in light of its physiology, life history, and the habitat changes occurring in St. Joseph Bay since August 1999. Our investigation is the first to report regional elimination of an octopus species in Florida and may provide useful insights into the effects of HABs on cephalopod populations.

METHODOLOGY

Historical densities and distributions of *Octopus* cf. *mercatoris* in St. Joseph Bay were determined from published accounts, pers. comm.s, and direct observations during collection trips made approximately one year prior to the HAB of 1999. The only published account quantifying the density of *O.* cf. *mercatoris* in St. Joseph Bay was reviewed (Mather 1982b) and other publications citing numbers of octopus captured from St. Joseph Bay (Emery 1975, Mather 1978, Mather 1982a, Cigliano 1995) were evaluated. The authors were interviewed for information regarding (1) approximate octopus densities or catch per unit effort during successful collection trips, (2) time of year collections were made, and (3) collection location. Unpublished accounts from local researchers, residents, and officials from St. Joseph Peninsula State Park augmented published data of occurrences of octopus. Finally, pre-HAB captures of octopus by the authors for use in studies of metabolic rate were used to define the last known pre-HAB collection date.

Post-HAB searches for *Octopus.* cf. *mercatoris* were made at monthly intervals from September 1999 through October 2002 and every 3 months from October 2002-February 2004. Two swimmers using snorkeling gear typically carried out searches in seagrass beds <2 m deep, although for some searches as many as seven snorkelers were present. All search teams included at least one swimmer experienced in searching for octopuses. Known areas of previous high-density occupation by octopus, *i.e.*, beds of the pen shell, *Atrina rigida* (Lightfoot, 1786), located on the northwest corner of Black's Island, as well as habitat 600 m northwest and 600 m southwest of the Eagle Harbor boat landing, were thoroughly searched at two month intervals over the 4½ year study period. In addition to previous known habitat, other sites with high densities of pen shell located along the Bay margins were searched for octopus (Fig. 1). Although more than one of these other pen shell sites may have been searched during any given trip, each site was generally searched only once per year. Areas smaller than 400 m^2 were searched in their entirety, whereas at larger sites, two to four randomly selected 400 m^2 plots were systematically searched. Empty bivalve shells (mostly pen shells, *Atrina* spp.), gastropod shells, bottles, and other debris of suitable size and shape within the search area were carefully inspected for the presence of *O. cf. mercatoris.* Because these animals typically camouflage themselves with sand, small shells, and bits of debris to avoid detection, all structures were completely emptied of contents during inspection. On average, two to three sites and 400 or more structures (shells, bottles, etc.) were inspected during a 5-7 hour search period.

We examined pertinent life history strategies and relevant abiotic habitat conditions dominating the bay system to assess the potential for re-colonization by *Octopus* cf. *mercatoris* in St. Joseph Bay. Previously published descriptions of the early life history of *O.* cf. *mercatoris* (Forsythe and Toll 1991), as well as direct observations of animals raised from eggs in the Ecological Physiology Laboratory at the University of West Florida were used in the assessment. Relevant life history characteristics were then evaluated in conjunction with changes in patterns of land use and development in and around St. Joseph Bay, and with data on

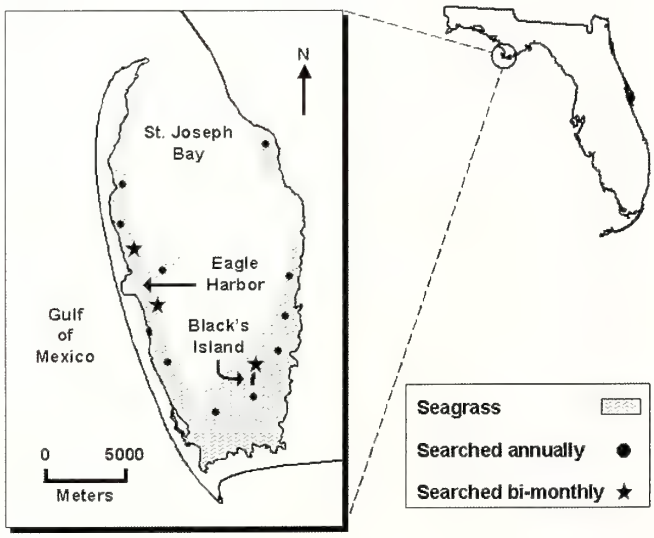

Figure 1. Map of St. Joseph Bay, Bay County, Florida, U.S.A., showing sites searched for *Octopus cf. mercatoris* between August 1999 and March 2004. Stars depict sites at which *Octopus cf. mercatoris* have been historically abundant [Source: NOAA nautical chart: State of Florida, Apalachicola Bay to Cape San Blas].

recent trends in HAB frequency and severity in Florida provided by the Fish and Wildlife Research Institute.

RESULTS

The two female specimens captured on 4 April 1999 (600 m northeast of Eagle Harbor) and 1 March 2003 (Black's Island) successfully reared eggs in our laboratory. The relatively large eggs (6.08 ± 0.325 mm) and benthic hatchlings confirmed the findings of Forsythe and Toll (1991) that the species of shallow-water octopus from St. Joseph Bay were *Octopus* cf. *mercatoris*. Data from published accounts and personal communications of collections prior to the August 1999 HAB suggest that *O.* cf. *mercatoris* were not distributed evenly throughout St. Joseph Bay (Table 1). Most accounts report that individuals of *Octopus* cf. *mercatoris* were collected along the southwest shoreline of St. Joseph Bay approximately 600 m southwest or northwest of Eagle Harbor (Fig. 1) (J. Hemming pers. comm., C. D'Asaro pers. comm.) and in areas near Black's Island (Emery 1975, Mather 1978, Mather 1982b, Cigliano 1995). Other areas of St. Joseph Bay may be less amenable to colonization by *O.* cf. *mercatoris*. Neither the very shallow beds (<0.5 m in many areas) located at southeast end of the Bay nor the areas near the mouth, which are frequently dredged, support the growth of seagrasses.

A review of pre-HAB studies suggests that *Octopus* cf. *mercatoris* has highly variable seasonal and spatial densities in St. Joseph Bay. Although individuals of *O.* cf. *mercatoris* were collected during every season, the highest numbers of animals were collected during summer and early fall when water temperatures were warm and newly-hatched individuals had reached noticeable size (approximately 25-30 mm mantle length). Lower sampling effort during colder months probably resulted in lower numbers for winter collections. The most frequented collection site was Black's Island where Mather (1982b) made the only direct measurements of densities of *O.* cf. *mercatoris* in the Bay during October 1979 and reported an average density of one octopus per 33 m^2. Indirect estimates of density based on catch-per-day efforts of five researchers over multiple collection trips between 1972 and 1990 ranged between 1 and 70 octopus/day (Table 1). In general, collections were made over irregular intervals and likely did not impact octopus populations; none of the researchers contacted reported negative searches. Most assessments were made at Black's Island; however, collections at Eagle Harbor, while less frequent, also produced large numbers of octopus in single-day collections (Table 1).

Prior to the 1999 HAB, on 25 June 1999, we captured 5 juvenile *O.* cf. *mercatoris* residing in empty pen shells at the north end of Black's Island. Monthly post-HAB searches of areas where *O. cf. mercatoris* has historically been present (*i.e.*, Black's Island and Eagle Harbor), as well as searches of several other seagrass/pen shell assemblages, resulted in the observation of only a single brooding female on 1 February 2003 (Table 1). The relatively large egg size and location near Black's Island suggested that the female was *O.* cf. *mercatoris*; however, we did not disturb the octopus or clutch to obtain exact measurements. In addition, park personnel reported that they had been unable to locate a specimen *O.* cf. *mercatoris* for their public display tank since the 1999 HAB

Table 1. Published records and presonal communications estimating numbers of *Octopus* cf. *mercatoris* captured from St. Joseph Bay between 1972 and 2003. Values are expressed as density per m^2 or catch-per-day. Data represent collections from various locations.

Collection date	Location	Number collected	Reference
Pre-harmful algal bloom			
October 1972	Black's Island	38, single day	Mather 1982a
1973	Unknown	>1	Emery 1975
January 1977	Black's Island	1 brooding female	Forsythe 1984
August 1977	Black's Island	6, single day	Mather 1978
October 1979	Black's Island	70, single day	Mather 1982a
1980-1991	Black's Island	20 to 30 per day	J. W. Forsythe pers. comm.
1980-1997	600 m SW of Eagle Harbor	Common	C. D'Asaro pers. comm.
October 1982	Black's Island	1 per 29.4 m^2, in seagrass 1 per 138 m^2, in sand	Mather 1982b
1993	Unknown	15	Cigliano 1995
August 1994	600 m NW of Eagle Harbor	80, two days	J. Hemming pers. comm.
April 1999	600 m NW of Eagle Harbor	1 brooding female	Present study
June 1999	Black's Island	5, single day	Present study
Post-harmful algal bloom			
February 2003	Black's Island	1 brooding female	Present study

event, and had received no reports of sightings by persons visiting the park. At one time sightings were weekly occurrences (T. Summers pers. comm.). Interviews conducted with local fishermen and tourists have yielded similar negative results.

DISCUSSION

Ubiquitous in seagrass sites prior to the summer of 1999, *Octopus* cf. *mercatoris* has been virtually absent from St. Joseph Bay since at least 25 June 1999. Exhaustive monthly efforts to locate the octopus have found only one brooding female in February 2003 leading us to conclude that the octopus occurs in numbers so low that it may be unable to repopulate its former range and is in danger of disappearing. Published accounts, coupled with anecdotal reports from researchers, park officials, and local residents, suggest a continuous record of occurrence of *O.* cf. *mercatoris* in St. Joseph Bay for at least the last 32 years. It is likely that this species had been common to the Bay much longer than the literature records. Populations of *O.* cf. *mercatoris* have been confirmed in only three other Florida locations (Forsythe and Toll 1991), and its loss in St. Joseph Bay would seriously deplete the known Florida population. Other populations may exist in Florida, but may have been misidentified as *O. joubini* (Forsythe and Toll 1991).

While the specific reasons behind the disappearance of *Octopus* cf. *mercatoris* from St. Joseph's Bay may never be known with certainty, the occurrence of the 1999 HAB was likely an important contributing factor in their disappearance. Prior to August 1999 cell counts of *Karenia brevis* were below 10,000 cells/L. During the HAB event, however, cell counts of more than 1,000,000 cells/L were reported (Fish and Wildlife Research Institute 2004b). The event killed thousands of menhaden, *Brevoortia patronus* (Goode, 1878); redfish *Sciaenops occelatus* (Linnaeus, 1766); hardtails, *Caranx crysos* (Mitchill, 1815) and pinfish, *Lagodon rhomboides* (Linnaeus, 1776); as well as hundreds of horseshoe crabs, *Limulus polyphemus* (Linnaeus, 1857), and blue crabs, *Callinectes sapidus* (Rathbun, 1896) (N. A. Fangue, pers. obs.). Roberts *et al.* (1979) documented 17 common invertebrates that were absent immediately following a HAB in Tampa Bay in 1975. Molluscan species included the banded tulip, *Fasciolaria lilium hunteria* (Perry, 1811); crown conch, *Melongena corona* (Gmelin, 1791); and lettered olive, *Oliva sayana* (Ravenel, 1834) (Florida Marine Research Institute 2004b). Cephalopods are not usually listed among animals killed during HAB events, perhaps due to their overall lower population levels, or to the fact that most do not leave behind shells that are easily observed or counted. However, approximately ten dead octopuses (species unknown) were observed near Presnell's Marina on the northeast shore of St. Joseph Bay's during the 1999 HAB (E. Sander pers. comm.).

During a HAB event, individuals of *Octopus* cf. *mercatoris,* which are already coping with abiotic extremes imposed by their environment, may be readily victimized by the added physiological stress associated with the bloom. Some larger octopus of other species have been reported to abandon their dens during hypoxic events (Stiffler 2003). The extensive nature of the 1999 HAB, however, likely denied individuals of *O.* cf. *mercatoris* access to more amenable habitats, and it is unlikely that these small molluscs could have migrated 20 km to the mouth of St. Joseph Bay. The harmful effects of a bloom of *Karenia brevis* on octopus physiology are potentially twofold. Brevetoxins released by *K. brevis* have been shown to produce neurological impairment in fish, resulting in paralysis and respiratory dysfunction, ultimately leading to death (Quick and Henderson 1974). Similar responses may occur in the cephalopod nervous system as well. In addition, accelerated oxygen demand of a logarithmically increasing population of *K. brevis* combined with elevated bacterial activity on decomposing animals killed by the bloom can reduce dissolved oxygen below detectable levels, suffocating active fishes and invertebrates (Fish and Wildlife Research Institute 2004a). Cephalopods regulate oxygen uptake over a wide range of oxygen tensions by increasing cardiac and ventillatory stroke volume and ventilation frequency (Maginnis and Wells 1969). This strategy is metabolically expensive and may not be effective in situations where brevetoxins compromise neurological control, or if hypoxic conditions are as prolonged as often occurs during a HAB event (Fish and Wildlife Research Institute 2004a). The situation may be worsened by the inherent tendency of individuals of *O. cf. mercatoris* to remain in their shelters rather than attempt to escape to more amenable conditions.

Opportunities for remaining individuals of *Octopus* cf. *mercatoris* to re-colonize St. Joseph Bay may be severely diminished by future HAB events and/or alteration of critical habitat. Although harmful algal blooms are well-documented in the Gulf of Mexico, dating back to the 1500s (Fish and Wildlife Research Institute 2004b), they may not have posed as serious a problem to octopus in the past. From 1878-1972 harmful algal blooms were seen in the Gulf of Mexico an average of 2.96 months/year. Since 1972, however, this average has increased to 4.78 months/year (Fish and Wildlife Research Institute 2004b). The pattern of HAB occurrence in the Gulf of Mexico is not unique. Indeed, HABs seem to be increasing in frequency, duration, and severity worldwide (Hallegraeff 1993, Smayda and White 1990, Van Dolah 2000). Consequently, octopus populations may have little reprieve from recurrent, severe HAB episodes. In addition, land use around St. Joseph Bay is

changing rapidly. Thousands of acres of previously untouched property near and around the Bay were sold to developers in late 1999. Even Black's Island, which at one time was uninhabited, is now being cleared for development of residential homes. The resulting increase in boat traffic, foot traffic, and eutrophication may negatively impact the seagrass/pen shell habitats preferred by *O.* cf. *mercatoris*. The combination of increasing anthropogenic insult, recurrent perturbation of water quality and octopus life history suggest that population levels of this small mollusc may never return to historic levels and may be at high risk for regional extinction.

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LITERATURE CITED

Anderson, D. M. 1994. Red tides. *Scientific American* [August 1994]: 62-68.

Cigliano, J. A. 1995. Assessment of the mating history of female pygmy octopuses and a possible sperm competition mechanism. *Animal Behavior* **49**: 849-851.

Emery, D. G. 1975. Ciliated sensory cells and associated neurons in the lip of *Octopus joubini* Robson. *Cell and Tissue Research* **157**: 331-340.

Fangue, N. A. and W. A. Bennett. 2003. Thermal tolerance responses of laboratory- acclimated and seasonally-acclimatized Atlantic stingray, *Dasyatis sabina*. *Copeia* **2003**: 315-325.

Fish and Wildlife Research Institute. 2004a. The effects of harmful algal blooms on marine animals. Available at: http://floridamarine.org/features/view_article.asp?id=5964 19 May 2004.

Fish and Wildlife Research Institute. 2004b. Harmful algal blooms historical events. Available at: http://floridamarine.org/features/view_article.asp?id=1680 19 May 2004.

Forsythe, J. W. 1984. *Octopus joubini*: A detailed study of growth through the full life cycle in a closed seawater system. *Journal of Zoology London* **202**: 393-417.

Forsythe, J. W. and R. B. Toll. 1991. Clarification of the western Atlantic Ocean pygmy octopus complex: The identity and life history of *Octopus joubini* (Cephalopoda: Octopodinae). *Bulletin of Marine Science* **49**: 88-97.

Hallegraeff, G. M. 1993. A review of harmful algal blooms and their apparent global increase. *Phycology* **322**: 311-324.

Harrington, R. W. Jr. and E. S. Harrington. 1961. Food selection among fishes invading a high subtropical salt marsh from onset of flooding through the progress of a mosquito brood. *Ecology* **42**: 646-666.

Maginnis, L. A. and M. J. Wells. 1969. The oxygen consumption of *Octopus cyanea*. *Journal of Experimental Biology* **51**: 607-613.

Mather, J. 1978. Mating behavior of *Octopus joubini* Robson. *The Veliger* **21**: 265-267.

Mather, J. 1982a. Choice and competition: Their effects on occupancy of shell homes by *Octopus joubini*. *Marine Behavior and Physiology* **8**: 285-293.

Mather, J. 1982b. Factors affecting the spatial distribution of natural populations of *Octopus joubini* Robson. *Animal Behavior* **30**: 1166-1170.

Pickford. G. E. 1945. A review of the littoral cephalopods from central and western Atlantic stations in the collections of the British History Museum. *Annals and Magazines of Natural History* **13**: 412-429.

Roberts, B. S. A., G. E. Henderson, and R. A. Medlyn. 1979. The effect of *Gymnodinium breve* toxin (s) on selected mollusks and crustaceans. *In*: D. L. Taylor and H. H. Seigler, eds., *Toxic Dinoflagellate Blooms*. Elsevier, North Holland. Pp. 419-424.

Robson, G. C. 1929. *A Monograph of the Recent Cephalopoda*, Part 1. Octopodinae. British Museum (Natural History), London.

Roper, C. F. E., M. J. Sweeney, and C. E. Nauen. 1984. Species catalogue. Cephalopods of the World. An annotated and illustrated catalog of species of interest to fisheries. *Fisheries and Agricultural Organization of the United Nations Fisheries Synopsis* **125**: 1-277.

Stiffler, L. 2003. Hood canal marine life struggling for oxygen. Seattle Post Intelligencer. http://seattlepi.nwsource.com/local/139800_hood16.html 30 June 2004.

Quick, J. A. and G. E. Henderson. 1974. Behavioral, hematological and histological evidences of new icthyointoxicative mechanisms in *Gymnodinium breve* red tides. *In: Proceedings of the Florida Red Tide Conference 10-12 October 1974*. Florida Department of Natural Resources, Marine Research Laboratory, St Petersburg, Florida. Pp. 8-11.

Smayda, T. J. and A. W. White. 1990. Has there been a global expansion of algal blooms? If so is there a connection with human activities? *In*: E. Granelli, ed., *Toxic Marine Phytoplankton*. Elsevier, New York. Pp. 516-517.

Van Dolah, F. M. 2000. Marine algal toxins: Origins, health effects and their increased occurrence. *Environmental Health Perspectives* **108**: 133-141.

Voss, G. L. 1956. A review of the cephalopods of the Gulf of Mexico. *Bulletin of Marine Science of the Gulf and Caribbean* **6**: 85-178.

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A new species of *Sonorella* (Pulmonata: Helminthoglyptidae) from Arizona, with notes on predation and evasive behaviors

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Abstract: A new species of *Sonorella* Pilsbry, 1900, from the Pedregosa Mountains of southeastern Arizona USA, is described. It shows many similarities to *Sonorella binneyi* Pilsbry and Ferriss, 1910. The desert box turtle, *Terrapene ornata luteola* Smith and Ramsey, 1952, is a predator of the snail.

Key words: box turtle, land snail, Pedregosa Mountains

The genus *Sonorella* Pilsbry, 1900, is comprised of numerous species of medium to large (13-30 mm diameter) land snails. They inhabit a five state area of the southwestern U.S. and northern Mexico, reaching their greatest diversity in the mountain ranges of southeastern Arizona, including the massive Chiricahua Mountains. At present, there are seven described species of *Sonorella* from this range and its northern extension, the Dos Cabezas Mountains (Bequaert and Miller 1973).

The new species, described herein, is found in the Leslie Canyon National Wildlife Refuge located in the Pedregosa Mountains, a southwestern extension of the Chiricahuas. It was discovered by WRR on 18 September 2000 following an overnight summer rainstorm when an individual was observed crawling in a funnel trap made of fine wire mesh used to monitor amphibians and reptiles (Manley and Radke 2002).

MATERIALS AND METHODS

Active specimens of the new species were hand-collected in the field by WRR following summer monsoonal rains (primarily 10 August 2002), and by both authors, along with Jacob Malcom, on 24 February 2005, after a series of heavy winter rains. Numerous shells were collected in talus accumulations at various other times when live specimens could not be found (primarily 10 April 2001). In addition, three estivating topotypes of *Sonorella binneyi* Pilsbry and Ferriss, 1910, were collected by LHG and Bobby Ray Holroyd Jr. on 2 April 2003.

For descriptive and comparative studies of the reproductive organs, eleven specimens of the new species and the three specimens of *Sonorella binneyi* were drowned, removed from their shells, and dissected. Their reproductive systems were separated from the other organs, stained with Delafield Hematoxylin, counterstained with Eosin B, and mounted on slides for microscopic examination (see Gregg 1959, Naranjo-García 1989). It was then determined that seven specimens of the new species and one specimen of *S. binneyi* were sexually mature.

Radulae were prepared for scanning electron microscopy by removal from the buccal mass and subsequent attachment to copper coins using double-sided adhesive tape. The coins were mounted on SEM stubs using carbon-adhesive tabs and sputter-coated with gold-palladium. Shells were cleaned in an ultrasound chamber and mounted directly on SEM stubs with clay and carbon-adhesive tabs prior to sputter-coating. Micrographs were taken with a Hitachi S-3000N scanning electron microscope.

The taxonomy used herein follows Turgeon *et al.* (1998). For the genus *Sonorella*, Turgeon *et al.* (1998) rely heavily on Bequaert and Miller (1973), which, in turn, modifies and elaborates on Pilsbry (1939). Supraspecific phylogeny-based taxa are from Roth's (1996) cladistic analysis of the family Helminthoglyptidae.

Abbreviations of institutions referred to in this article are as follows: ANSP, Academy of Natural Sciences of Philadelphia; CNMO, Colección Nacional de Moluscos (Mexico); LACM, Natural History Museum of Los Angeles County; SBMNH, Santa Barbara Museum of Natural History; USNM, National Museum of Natural History - Smithsonian Institution.

PREDATION AND EVASIVE BEHAVIORS

Predation. The desert box turtle (*Terrapene ornata luteola* Smith and Ramsey, 1952) is a significant predator of the new species of *Sonorella*, described herein. On the morning of 12 August 2002, after an overnight monsoonal rain-

storm measuring 12.5 mm, one was observed in the process of eating an active snail by WRR. The turtle was collected and its feces were found to be full of shell fragments of the new species. It is believed that this is the first account of a *Sonorella* being eaten by a box turtle.

Evasive behaviors: Captive snails were occasionally observed to exhibit evasive behaviors when disturbed (prodded), especially upon emerging from estivation (LHG). On certain occasions, when prodded, a snail would initially crawl two to three times faster than normal and then rotate its shell rather rapidly (for a snail), giving the visual effect of swinging it back and forth. One snail was observed to exude orange-colored mucus during its initial flight reaction. This snail then arched its body upward, crawled with a lurching motion, and began rotating (swinging) its shell. These behaviors lasted for about 20-30 seconds.

Subsequently, on the morning of 29 July 2004, following overnight rains of 9.5 mm at the type locality, several active, adult snails were located, and similarly prodded by WRR. About one in three specimens exhibited evasive behaviors like those described above, especially with regard to the discharge of orange mucus and the rapid shell rotation (the snails appeared to wiggle).

SYSTEMATICS

Superfamily HELICOIDEA
Family HELMINTHOGLYPTIDAE Pilsbry, 1939
Clade Sonorellamorpha Roth, 1996
Clade Sonorellales Roth, 1996
Genus *Sonorella* Pilsbry, 1900
Sonorella pedregosensis sp. nov.
(Figs. 1-5A; Tables 1, 2)

Diagnosis: A relatively small *Sonorella* with a depressed-globose, umbilicate shell. Male genitalia exhibit a long, coiled epiphallus, and a smooth, moderately thick verge with an apical, heart-shaped, glandiform structure.

Body coloration: Tentacles black; body integument gray-brown (occasionally black) with narrow, tan, mid-dorsal stripe. Mantle collar slightly off-white, sometimes with yellowish tinge; pallial membrane unpigmented. Foot tan.

Description of shell of holotype (Fig. 1; Table 1): Shell comparatively small in size (diameter 17.8 mm, height 11.0 mm), with 4.5 whorls, depressed globose, umbilicate (umbilicus 2.2 mm diameter, contained about 10 times in the major diameter of the shell), rather thin/translucent, medium tan in color with a silky-lustrous periostracum. Embryonic whorls 1.7 in number; first half-turn with ripples followed by two-thirds turn with spirally descending and ascending threads, some with cross-threads between them. Neanic whorls exhibit numerous collabral growth striae and a wide (1.4 mm), reddish-brown shoulder band on last 2.4 whorls (narrowly visible above suture on last 0.4 of antepenultimate and first 0.7 of penultimate whorls). Aperture large, oblique, rounded, slightly wider than high, margins converging. Parietal callus rather thin. Peristome slightly expanded; columellar lip elongated forward and reflected, partially covering umbilicus (approximately 10%).

Paratypes (Fig. 2, Table 1): Ten representative paratypes range from 16.5 × 10.2 to 18.1 × 11.8 with a mean of 17.5 × 11.0 (diameter × height in mm). There is some variation with regard to the strength and number of the apical threads. Some shells have a simple peristome while a few others have a more thickened one than holotype.

Description of jaw and radula (Fig. 3): Jaw with 6-7 moderate-sized ribs (based on observations of 3 jaws).

Radulae somewhat variable; one specimen with 83 teeth per row (41-1-41), another with 77 (38-1-38). Central and

Figure 1. *Sonorella pedregosensis* Gilbertson and Radke, sp. nov., holotype: apical view (left), apertural view (middle), umbilical view (right). LACM 3046. Shell 17.8 × 11.0 mm.

Table 1. Measurements (mm) of holotype (LACM 3046) and representative paratypes of *Sonorella pedregosensis* Gilbertson and Radke, sp. nov. Mean values are for paratypes only.

Specimen	# of whorls	Shell diameter	Shell height	Aperture	
				Width	Height
Holotype	4.5	17.8	11.0	10.0	8.7
Paratypes	4.4	18.0	11.9	9.6	8.2
	4.2	17.8	10.8	9.9	8.2
	4.2	16.5	10.2	9.0	8.1
	4.5	18.0	10.8	10.2	8.6
	4.7	16.8	10.9	9.1	8.1
	4.5	17.6	11.3	9.8	8.2
	4.4	18.1	11.8	10.5	9.0
	4.6	17.1	11.0	9.1	8.5
	4.5	17.7	11.4	9.6	8.6
	4.5	17.4	10.2	9.8	8.5
$\bar{x}$	4.5	17.5	11.0	9.7	8.4

lateral teeth with bluntly pointed cusps; central tooth slightly smaller than laterals. Ectocone formation gradual, beginning on approximately the 12th tooth; mesocones of above the 17th tooth becoming bifid. Marginal teeth have blunt, bifid mesocone; occasional teeth show bifid ectocones. Outer 3-4 marginals have shorter cusps than previous teeth, most with bifid ectocones.

Description of reproductive anatomy of holotype (Fig. 4; Table 2): Description based on stained, slide-mounted, illustrated preparation of holotype. Hermaphroditic duct, albumen gland, and uterus typical in appearance; vagina slightly more than half the length (0.6) of penis. Spermathecal duct moderately long, unbranched; spermatheca rather large, round. Penis fairly large in size with numerous, finely corrugated, internal rings of tissue (presumably glandular) in proximal half. Verge slightly more than half the length of penis, moderately thick (1.0 mm in diameter), relatively smooth, exhibiting a rounded, heart-shaped, glandiform structure at apex. Cleavage of "heart" leading to a short, central, bluntly rounded protrusion (shown as a short line on figure). Opening of seminal duct on dorsal surface of central protrusion with slit continuing proximally along left edge of cleavage; shorter secondary or vestigial slit along right edge. Penial lumen surrounds verge, clearly separating it from inner wall of penis. Penial sheath envelops lower half of penis. Epiphallus coiled and elongate, approximately twice length of penis; proximal region slightly enlarged and connected to penial sheath by broad band of connective tissue; short distal section embedded in penial retractor muscle. Epiphallic cecum comparatively long (about 1.5 mm), detached. Penial retractor muscle short (contracted), inserting on epiphallus noticeably above (1.6 mm) apex of penis and continuing to apex.

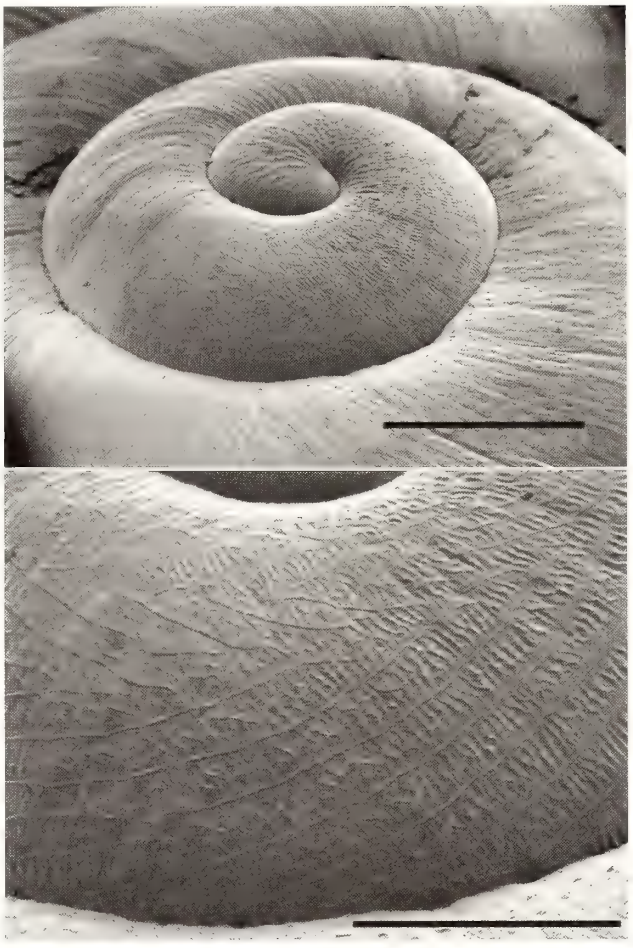

Figure 2. Scanning electron micrographs of the apical whorls of the shell of *Sonorella pedregosensis* Gilbertson and Radke, sp. nov., paratype. Oblique view and enlargement. LACM 3047. Scale bars = 1 mm (upper), 500 μm (lower).

Paratypes (Table 2, Fig. 5A): Paratypes similar to holotype; most specimens somewhat smaller overall. Seminal duct opening more visible (slit more opened) atop central protrusion on some stained paratypes compared to holotype. Largest paratype (no. 6) removed from snail with below average sized shell (Table 1, no. 10).

Type locality: Arizona, Cochise County, Pedregosa Mountains, Leslie Canyon National Wildlife Refuge (a unit of the San Bernardino National Wildlife Refuge Complex), 26 km N of Douglas, WNW facing talus slope, 31°35.526'N; 109°30.395'W. Elevation 1,421 m.

Leslie Canyon NWR was established to protect wetland habitat for federally listed endangered fishes. It is located at an interface between the Pedregosa and Swisshelm Mountains, characterized by both rhyolite and limestone formations. Live specimens of *Sonorella pedregosensis* and/or shells

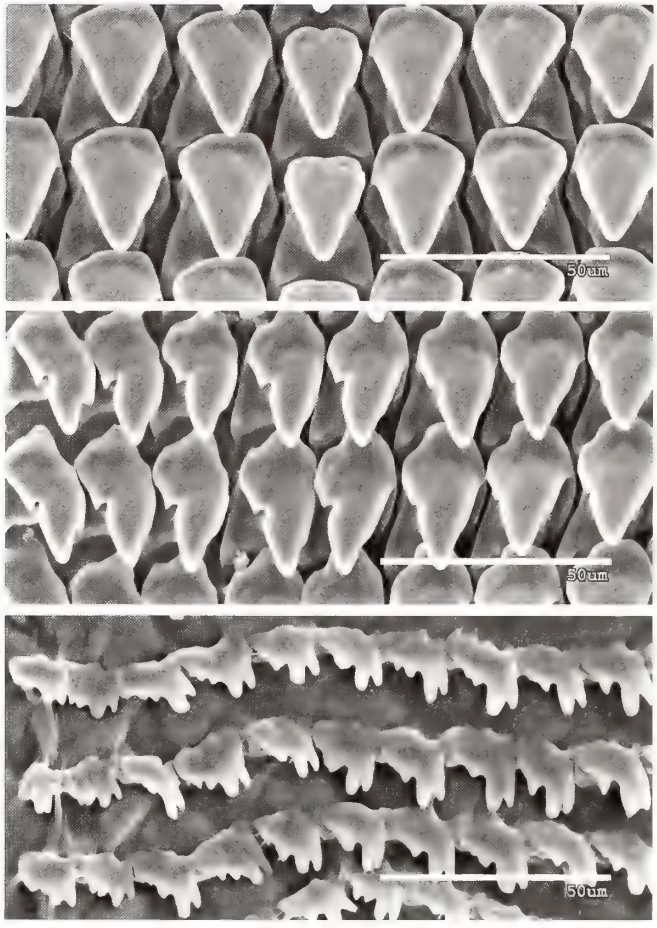

Figure 3. Scanning electron micrographs of radulae of *Sonorella pedregosensis* Gilbertson and Radke, sp. nov., paratypes. Upper image, central tooth and adjacent lateral teeth; middle image, transitional lateral-marginal teeth, numbers 11-18 (right to left); lower image, outer marginal teeth, numbers 32-41 (right to left). Scale bars = 50 μm.

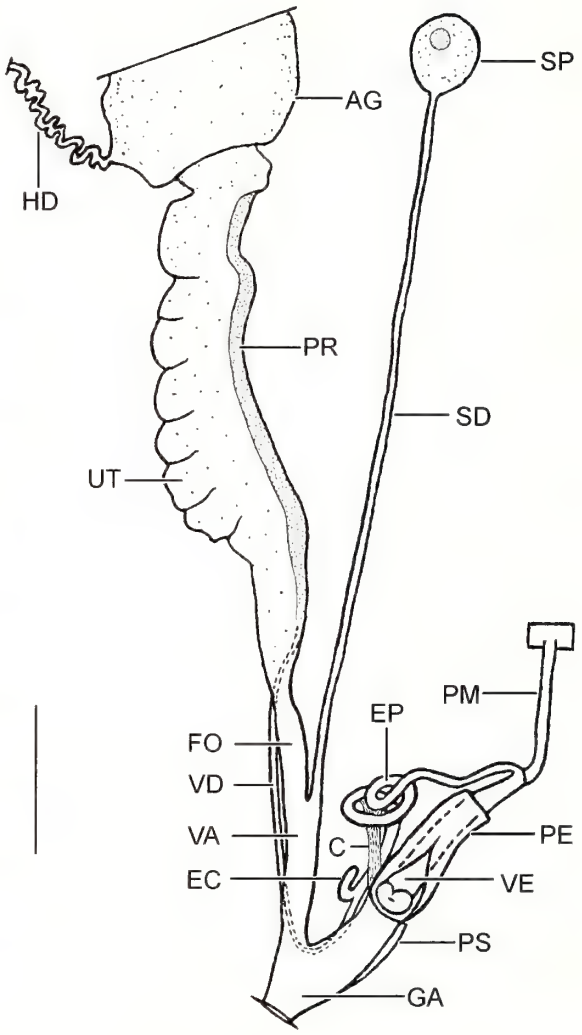

Figure 4. Drawing of the slide-mounted reproductive system of *Sonorella pedregosensis* Gilbertson and Radke, sp. nov., holotype (LACM 3046). Penis partially opened to expose verge. Abbreviations: AG, albumen gland; C, connective tissue; EC, epiphallic cecum; EP, epiphallus; FO, free oviduct; GA, genital atrium; HD, hermaphroditic duct; PE, penis; PM, penial retractor muscle; PR, prostate gland; PS, penial sheath; SD, spermathecal duct; SP, spermatheca; UT, uterus; VA, vagina; VD, vas deferens; VE, verge. Scale bar = 5 mm.

have been found among rhyolite talus at several locations on the refuge. The area is characterized by Chihuahuan Desert scrub bisected by riparian woodland. Dominant vegetation at the collection site is composed of *Fraxinus velutina*, *Jugulans major*, *Rhus microphylla*, *Sporobolus airoides* var. *Wrightii*, *Celtis reticulata*, *Acacia constricta*, and *Agave palmeri*. Average annual precipitation at Leslie Canyon is about 276 mm with rainfall occurring primarily during summer monsoonal thunderstorms (measured on-site by refuge staff using an "All Weather Gauge"). Temperature extremes for the area range from –10°C to 42°C (National Climate Data Center, Douglas FAA Airport location, station number 022664). Collecting is prohibited in the Leslie Canyon NWR without a Special Use Permit.

Disposition of types: Holotype (shell and slide of reproductive anatomy): LACM 3046. Paratypes: ANSP 412176 (2 shells); CNMO 1440 (2 shells); LACM 3047 (2 shells, 3 slides of reproductive anatomies), 3048 (6 shells); SBMNH 354176 (2 shells), 354183 (3 slides of reproductive anatomies); USNM 1073075 (2 shells).

Etymology: This species is named for the Pedregosa Mountains where it lives. For purposes where a common name is useful, the "Leslie Canyon talussnail" is proposed.

Discussion: *Sonorella pedregosensis* resembles *Sonorella*

Table 2. Lengths (mm) of selected reproductive organs of *Sonorella pedregosensis* Gilbertson and Radke, sp. nov., (LACM 3046,3047 [holotype, paratypes 1-3], SBMNH 354183 [paratypes 4-6]), and topotypes of *Sonorella binneyi*. Mean values for *S. pedregosensis* are for paratypes only. Abbreviation: sp./duct, spermatheca and spermathecal duct.

S. pedregosensis	Penis	Verge	Sheath	Vagina	Sp./duct
Holotype	8.3	4.6	4.0	4.8	29.0
Paratypes	7.0	4.3	3.5	5.4	–
	7.0	4.1	2.8	5.1	29.0
	7.5	4.2	2.9	5.8	21.8
	6.9	4.0	2.7	5.1	28.0
	7.2	4.4	2.7	5.6	31.1
	8.8	5.0	4.1	5.5	26.2
$\bar{x}$	7.4	4.3	3.1	5.4	27.2
S. binneyi					
LACM 153522	12.5	6.7	6.6	10.0	24.6
4802-A*	16.0	7.5	–	12.0	–
4910-A*	15.0	7.5	–	12.0	–
$\bar{x}$	14.5	7.2	6.6	11.3	24.6

* Miller 1967a,b (b, verge only)

binneyi Pilsbry and Ferriss, 1910 from Horseshoe Canyon, located on the eastern side of the Chiricahua Mountains. Like *S. binneyi*, the new species exhibits the hallmark characters of the "*S. binneyi* Complex Miller, 1967" of *Sonorella* species which include: (1) a relatively small, (comparatively) globose shell with a smooth, silky-lustrous periostracum and apical, spirally descending threads, and (2) a usually short, moderately thick, bluntly-rounded verge in the penis (see Pilsbry 1939, Miller 1967a, Bequaert and Miller 1973). Members of these two species also exhibit an unusually long, often coiled epiphallus, a long epiphallic cecum, and an initial insertion of the penial retractor muscle on the epiphallus noticeably above the apex of the penis. However, the shell of *S. pedregosensis* is somewhat smaller and darker and its shoulder band is darker and wider than that of *S. binneyi*. The apical whorls of *S. binneyi* exhibit spiral threads that are descending only. More significantly, the genitalia of *S. binneyi* are about twice as large as those of the new species (Table 2) and the glandiform apex of its verge includes the presence of a unique, lateral, subterminal, protrusible papilla with a terminal opening of the seminal duct (Miller 1967a,b, Fairbanks and Reeder 1980, Fig. 5B). (Pilsbry's [1939] original description of the verge was erroneous, apparently based on an immature specimen). Populations of these two species are separated by about 32 km of varied terrain.

The only other presently described Chiricahuan member of the "*Sonorella binneyi* Complex" is *Sonorella neglecta* Gregg, 1951. Its shell and genitalia are significantly smaller than those of the new species and they differ in other respects.

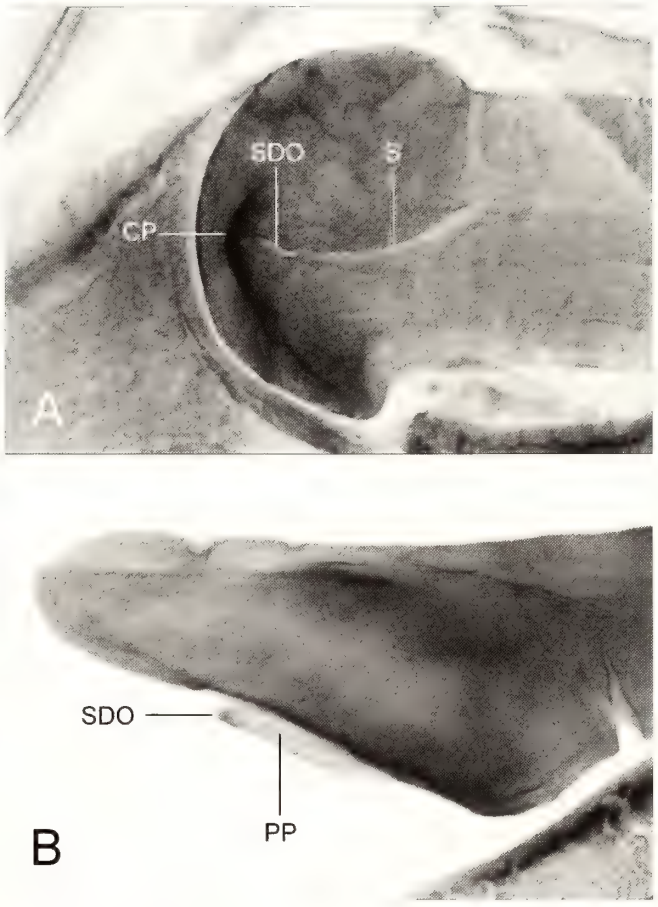

Figure 5. A, Photograph of the apical, glandiform structure of the verge of *Sonorella pedregosensis* Gilbertson and Radke sp. nov., paratype (Table 2, no. 6; SBMNH 354183) (Slit atypically along right edge of cleavage.) B. Photograph of the apical glandiform structure of the verge of *Sonorella binneyi* Pilsbry and Ferriss, 1910, topotype (LACM 153522). Both photographs are dorsal views with horizontal field widths of 2.7 mm. Abbreviations: CP, central protrusion; PP, protrusible papilla; S, slit; SDO, seminal duct opening.

Individuals of *Sonorella bowiensis* Pilsbry, 1905 from the northern end of the Chiricahua Mountains have shell features similar to those of *Sonorella pedregosensis*. However, the verge of this species lacks an apical, glandiform structure. It does have a subterminal seminal duct orifice (Miller 1967a). This species was placed by Pilsbry (1939) in his "*Sonorella binneyi* Group" and was subsequently transferred by Miller (1967a) to his reconfigured "*Sonorella granulatissima* Pilsbry, 1902 Complex."

The shell of *Sonorella pedregosensis* is rather similar to the shells of some other members of the "*Sonorella binneyi* Complex" inhabiting other mountain ranges to the west. These include *Sonorella tryoniana* Pilsbry and Ferriss, 1923 from the Patagonia Mountains, *Sonorella imperialis* Pilsbry

and Ferriss, 1923 from the Empire Mountains, and *Sonorella sitiens sitiens* Pilsbry and Ferriss, 1915 from the Las Guigas (and nearby) Mountains. However, the genitalia, especially the size and shape of the verge and the length of the epiphallus and epiphallic cecum, clearly identify the new species. Nominate *Sonorella baboquivariensis* Pilsbry and Ferriss, 1915 exhibits a glandiform structure at the tip of its verge but, because of its conic shape, it is easily separable from that of *S. pedregosensis*. In addition, its shell is more globose, more narrowly umbilicate, and the Baboquivari Mountains, where it lives, are located hundreds of kilometers to the west.

The validity of the informal *Sonorella* "species-groups" (or "complexes"), including the "*S. binneyi* Complex," has been brought into question by Naranjo-García (1988) and Roth (1996). Further research, including the use of molecular techniques, is needed to help clarify the relationships of these informal taxa.

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LITERATURE CITED

Bequaert, J. C. and W. B. Miller. 1973. *The Mollusks of the Arid Southwest, with an Arizona Check List,* University of Arizona Press, Tucson.

Fairbanks, H. L. and R. L. Reeder 1980. Two new species of *Sonorella* (Gastropoda: Pulmonata: Helminthoglyptidae) from the Pinalino Mountains, Arizona. *Proceedings of the Biological Society of Washington* **93**: 395-404.

Gregg, W. O. 1959. A technique for preparing in-toto mounts of molluscan anatomical dissections. *Annual Report of the American Malacological Union* **25**: 39.

Manley, D. G. and W. R. Radke. 2002. Synonomy of *Dasymutilla sicheliana* (Saussure) (Hymenoptera: Mutillidae). *Pan-Pacific Entomologist* **78**: 230-234.

Miller, W. B. 1967a. Anatomical revision of the genus *Sonorella* (Pulmonata: Helminthoglyptidae). Ph.D. Dissertation, University of Arizona, Tucson.

Miller, W. B. 1967b. Two new *Sonorella* from Sonora, Mexico. *The Nautilus* **80**: 114-119.

Naranjo-García, E. 1988. *Systematics and Biogeography of the Helminthoglyptidae of Sonora.* Ph.D. Dissertation, University of Arizona, Tucson. [University Microfilms International, Ann Arbor, Michigan: E9791 1988 272].

Naranjo-García, E. 1989. Four additional species of *Sonorella* (Gastropoda: Pulmonata: Helminthoglyptidae) from Sonora, Mexico. *The Veliger* **32**: 84-90.

Pilsbry, H. A. 1939. *Land Mollusca of North America (North of Mexico). Monographs of the Academy of Natural Sciences of Philadelphia* **3**,1: i-xvii, 1-573.

Roth, B. 1996. Homoplastic loss of dart apparatus, phylogeny of the genera, and a phylogenetic taxonomy of the Helminthoglyptidae (Gastropoda: Pulmonata). *The Veliger* **39**: 18-42.

Turgeon, D. D., J. F. Quinn, Jr., A. E. Bogan, E. V. Coan, F. G. Hochberg, W. G. Lyons, P. M. Mikkelsen, R. J. Neves, C. F. E. Roper, G. Rosenberg, B. Roth, A. Scheltema, F. G. Thompson, M. Vecchione, and J. D. Williams. 1998. *Common and Scientific names of Aquatic Invertebrates from the United States and Canada: Mollusks,* 2nd Ed. American Fisheries Society, Special Publication 26. Bethesda, Maryland.

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Indicators of physiological condition in juveniles of *Utterbackia imbecillis* (Bivalvia: Unionidae): A comparison of rearing techniques

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Abstract: Larvae of *Utterbackia imbecillis* normally undergo metamorphosis to the juvenile while attached to the gills or fins of a host fish; however, metamorphosis can also be induced in the laboratory in a modified cell culture medium. This study examined juveniles resulting from each of these rearing techniques to determine their relative physiological conditions. Juveniles reared *in vitro* grew more slowly and had higher mortality rates than did their fish-reared counterparts. Animals reared on their host fish accumulated triglycerides, cholesterol, glycogen, and protein during the parasitic metamorphic period. In contrast, animals reared *in vitro* showed an increase in the levels of triglycerides, but did not accumulate cholesterol, glycogen, or protein. These results suggest that fish-reared juvenile individuals of *U. imbecillis* are in more robust physiological condition than their *in vitro*-reared counterparts.

Key words: condition, mussel, glochidium, juvenile, in vitro

Unionid mussels possess a larval form referred to as a glochidium that, for most species, requires a parasitic phase to complete its development. Glochidia normally attach to the gills or fins of a host fish (Kat 1984) where they then undergo metamorphosis into the juvenile mussel. Following metamorphosis, the juvenile mussel excysts from its host (Waller and Mitchell 1989), drops to the substratum, and continues development to an adult mussel. With the advent of techniques for the *in vitro* culturing of juvenile mussels (Isom and Hudson 1982), it also has become possible to rear these animals without employing their host fish. This technique has been used to rear juveniles for toxicity testing (Keller and Zam 1991, McKinney and Wade 1996) and for experimental studies of their basic biology (Dimock and Wright 1993, Warren *et al.* 1995, Polhill and Dimock 1996, Dimock 2000).

Currently there is no generally accepted method for measuring the physiological condition of juvenile freshwater mussels. Growth and survival have been measured in response to differing diet regimes (Gatenby *et al.* 1996, 1997), but no biochemical techniques have been used to assess juvenile health. The identification of sublethal indicators of stress is necessary because changes in growth occur too slowly to allow rapid assessment of the mussels' health.

Lipids, such as triglycerides and cholesterol, have been shown to be important energy reserves in aquatic larvae (Fraser 1989, Lochmann *et al.* 1995, Palacios *et al.* 1999), and their levels may be used to measure physiological condition. A lipid-based measure of condition has several advantages. First, lipid content has been shown to increase during feeding and decrease during starvation in the larvae of some marine bivalves (Gallager and Mann 1986, Gallager *et al.* 1986). Second, indices of lipid condition appear to be good predictors of survival and egg viability (Ouellet *et al.* 1992, Palacios *et al.* 1999). Finally, lipid concentration is strongly correlated with other measures of physiological fitness in larval fish and aquatic invertebrates (Fraser 1989, Lochmann *et al.* 1995).

Glycogen, the primary storage form of carbohydrates in adult bivalves (Bayne *et al.* 1982, Naimo *et al.* 1998), has been used as an indicator of the nutritional status of various molluscs (Kleinman *et al.* 1996). Because the amount of glycogen is relatively easy to determine and responds quickly to changes in the environment, it is commonly assessed to determine physiological condition in adult unionid bivalves (Haag *et al.* 1993, Naimo and Monroe 1999, Patterson *et al.* 1999).

Protein generally is metabolized only after lipid and glycogen reserves have been depleted. Protein utilization has been shown to increase during stress in the soft-shelled clam, *Mya arenaria* (Linnaeus, 1758) (Grant and Thorpe 1991), and relative protein content has been used as a condition index in some adult bivalves (Mann and Gallager 1985, Gabbott and Peek 1991).

The present study measured overall growth and survival as well as lipid, glycogen, and protein levels in juvenile individuals of *Utterbackia imbecillis* (Say, 1829). The objective was to compare the survival, growth, and physiological condition of juveniles that had metamorphosed from encystment on host fish with those that had metamorphosed *in vitro* in a tissue culture protocol.

METHODS

Animals

Adults of *Utterbackia imbecillis* were obtained from Davis' Pond (Davidson, North Carolina, U.S.A.) and maintained in the laboratory until mature glochidia were present in the marsupia. Glochidia were removed from the outer demibranchs of 2 mussels, washed in sterile moderately hard EPA water, a standard formulation for reagent grade artificial pond water (Lewis *et al.* 1994), and pooled before use.

For *in vitro* rearing, glochidia were placed in 60 x15 mm culture dishes in 3 ml of a standard tissue culture medium (Isom and Hudson 1982, Dimock and Wright 1993) and maintained in a 5% CO_2 incubator at 21°C for 7 days. Metamorphosed juveniles were either used immediately or transferred to reusable stainless steel coffee filters that were used as growth chambers. The bottom of each coffee filter was covered with a shallow layer of autoclaved pond silt (< 63 µm) pipetted onto a Gelman glass fiber filter. The coffee filters were suspended in a plexiglass rack in 10 L of moderately hard EPA water in 40-L aquaria that were part of a temperature controlled, recirculating aquarium facility. Juveniles were fed a 1:1:1 mixture of live *Neochloris oleoabundans,* dehydrated *Chlorella* sp., and spray-dried *Schizochytrium* sp. at a concentration of 10^6 cells per ml twice daily. The juveniles were maintained at 21°C under a 12L:12D photoperiod.

For rearing on host fish, glochidia were placed into 2 L of aged tap water with vigorous aeration. Individual bluegill sunfish (*Lepomis macrochirus* Rafinesque, 1819) were then transferred to the tank with the glochidia for 1-2 min to allow attachment of mussel larvae to the gills and fins. Following glochidial infection, fish were housed at 21°C and fed daily with commercial fish food (Tetramin). Five days post infection, the fish were transferred to 20 L polyethylene funnels with aerated tap water maintained at 21°C. During the following 1-5 days, metamorphosed juveniles excysted from the fish and dropped into a collecting system at the bottom of the funnel, from which they were removed and were either used immediately or placed into the rearing chambers as described for the *in vitro*-reared animals. Both fish-reared and *in vitro* reared animals were fed the same diet. The procurement and handling of all fish followed the protocol (#A00-024; R. Dimock) approved by the Wake Forest University ACUC.

Growth and Survival

Ninety *in vitro*-reared juveniles that were 24 h post metamorphosis were placed individually in wells of 3, 96-well microtiter plates (30 animals/plate) held at 20°C. Each animal was placed in 300 µl of moderately hard EPA water that had 10^6 live *Neochloris oleoabundans* per ml. Every 48 h, 150 µl of water were removed and replaced with fresh algae in EPA water. Survival was assessed daily, with death defined as the absence of ciliary movement and a visible heart beat. Shell length (longest anterior-posterior dimension parallel to the hinge) was measured via digital image analysis (Macintosh Quadra, NIH image) at the beginning of the experiment and at days 7 and 14. The same procedure was repeated for 90 fish-reared juveniles that had come from the same initial stock of glochidia and had excysted from a host fish no more than 24 h prior to the start of the study. Repeated measures ANOVA was used to determine the effect of age and rearing technique on shell length. Survival of juveniles during the growth study was analyzed with the Cox Proportional Hazards Model.

Biochemical indicators

The concentrations of lipids, glycogen, and protein were determined for glochidia, 1-day-old juveniles, and 7-day-old juveniles. Glochidia refers to mature larvae that had been removed from the parental mussels immediately before use. The 1-day-old animals were either those that were 1 day post metamorphosis from the *in vitro* culture system or mussels that had excysted from the fish during the previous 24 h. All 7-day-old animals had been in the continuous flow juvenile rearing system for 7 days prior to analysis. For the lipid, glycogen, and protein analyses, samples of approximately 2000 animals, shells included, were placed into microcentrifuge tubes and their wet weights recorded. The average wet weight was 0.032 g for the biochemical tests. Due to the small size of the animals (300-450 µm), a large number of individuals was required for each assay. Four samples (~2000 juveniles/sample) from each of the two rearing techniques at the appropriate ages were collected for each biochemical indicator. These were then frozen at −80°C until the assays were conducted. For each biochemical indicator, a sample of moderately hard EPA water was used as a control.

Lipid Quantification

Lipids were extracted using a modification of the Bligh-Dyer procedure (Bligh and Dyer 1959). Each sample of glochidia or juvenile mussels was thawed and then sonicated in 800 µl of distilled water to which was added 1 ml of chloroform with stigmasterol (20 µg/ml) as an internal standard. Methanol (2 ml), chloroform (1 ml), and distilled water (1 ml) were then added in sequence, and the samples were mixed following the addition of each solvent. The samples were then centrifuged for 20 min at 2400 RPM. The first ml of the choloroform supernatant was drawn off for triglyceride analysis and the next 1 ml was removed for cholesterol analysis.

To measure triglycerides, the samples were evaporated to dryness under nitrogen, and then resuspended in 200 µl of

1% TritonX-100 in chloroform. The samples were dried under nitrogen again and 100 µl of distilled water were added. After vortexing, the vials were placed in a 37°C water bath for 15 min. Fifty µl of each sample were then pipetted into individual wells on a 96-well microtiter plate. The reagents for the colorimetric assay were then added following the assay guidelines (Boehringer Mannheim, Inc). The plate was read at 505 nm at 25°C.

To measure cholesterol levels the samples were dried under nitrogen and then dissolved in 100 µl of tetramethylammonium hydroxide-isopropanol (TMH-i). After heating at 80°C for 15 min, 100 µl of tetrachlorethylene were added. The samples were mixed, 200 µl of distilled water were added, and then the samples were centrifuged for 10 min at 2500 RPM. Fifty µl of each sample were placed into gas chromatography tubes. Separations were carried out on a gas chromatograph (Hewlett–Packard Model 402B) at a column temperature of 260°C, inlet temperature of 290°C, and detector temperature of 300°C. Data were analyzed using the MANOVA (SAS Institute, Cary, North Carolina, U.S.A.) procedure to determine the effect of age and rearing condition on total lipid content and then followed with individual ANOVAs to determine the effects of age and rearing condition on triglycerides and cholesterol separately.

Glycogen Quantification

The extraction and quantification of glycogen followed the procedure of Naimo *et al.* (1998). Thawed samples were sonicated for 10 s in 400 µl of 30% KOH and then placed in a 100°C bath for 20 min. After cooling on ice for 5 min, the samples were treated with 600 µl of 95% ethanol and were placed back in the 100°C bath for 15 min. The samples were then diluted with 5 ml of distilled water and mixed. Glycogen content was determined by placing 2-ml aliquots of the extracts into test tubes with 100 µl of 80% phenol, followed by 5 ml of reagent-grade concentrated H_2SO_4. After allowing 30 min for the samples to return to room temperature, 1-ml aliquots were read at 490 nm in a spectrophotometer. A glycogen stock solution (Type VII, *Mytilus edulis*, Sigma) was used to prepare calibration standards. Data were analyzed using a 2-way ANOVA (SAS Institute).

Protein Quantification

To measure protein levels, the water was drawn off the samples and 10 µl of protease inhibitors (AEBSF Protease Inhibitor Cocktail [Sigma] in phosphate buffered saline) were added. The tubes were then sonicated twice for 10 s. Protein concentration was determined with a dye-binding assay (Bradford 1976) using bovine serum albumin as a standard. Data were analyzed using a 2-way ANOVA (SAS Institute) to determine the effect of rearing condition on protein content.

RESULTS

Growth and Survival

Rearing technique had a significant effect on the growth of juveniles of *Utterbackia imbecillis* (Repeated Measures ANOVA, $F_{(1,16)} = 988$, $p < 0.001$). Although there was no difference in size (length) among animals from the two rearing techniques on the first day following excystment, at days 7 and 14 of development, fish-reared juveniles were larger ($p < 0.05$) than those reared *in vitro* (Fig. 1). Rearing technique also had a significant effect on the survival of juveniles of *U. imbecillis* held at the same temperature and oxygen tension (Cox Proportional Hazards Model, $p < 0.0001$). A hazard ratio of 3.5 indicated that *in vitro*-reared juveniles were 3.5 times more likely to die before the next sampling period than were those animals reared on host fish. Survivorship was greater for fish-reared mussels at all time periods examined. The time to 50% mortality for *in vitro*-reared animals was 10 days while that for the fish-reared animals was 14 days at 20°C (Fig. 2).

Lipids

Both age and rearing method had significant effects on the levels of triglycerides of larval and juvenile individuals of *Utterbackia imbecillis* (Table 1). Glochidia had a low triglyceride content compared to the juvenile stages (Fig. 3). Although animals from both rearing techniques showed an increase in triglyceride concentration at 1 day post metamorphosis, this increase was significantly higher ($p < 0.05$) in those animals reared on the host fish. There was a significant decrease in the triglyceride levels of 7-day-old animals compared to 1-day-old animals from both rearing techniques; however, the *in vitro*-reared juveniles experienced an 88.6% decrease, whereas the fish-reared juveniles exhibited a 73.9% decrease. At the end of 7 days of growth, fish-reared

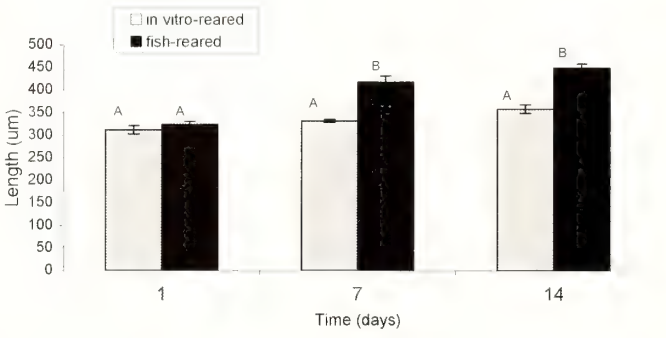

Figure 1. The effect of rearing technique on the growth of juveniles of *Utterbackia imbecillis*. Histograms indicate the mean length (± SE; initial N = 90) for each rearing condition. Bars labeled with the same letter are not significantly different ($p < 0.05$).

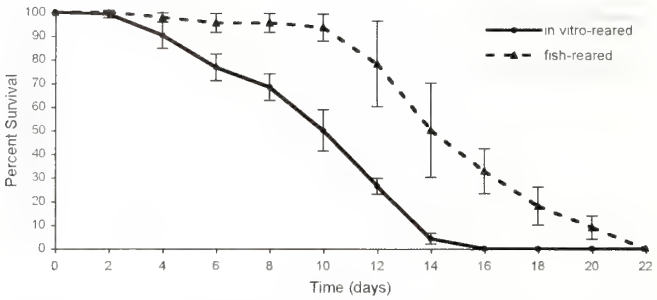

Figure 2. Survivorship curves for juveniles of *Utterbackia imbecillis* from the two different rearing techniques. Points are the mean (± SE) for an initial N = 30 for each of 3 replicates from each rearing technique.

Table 1. Results of 2-way ANOVA of the effects of age (glochidia, 1 and 7 d juveniles) and rearing condition (*in vitro* and fish-reared) on biochemical parameters of *Utterbackia imbecillis.*

Source	Df	MS	F	P
Triglyceride				
Age (A)	2	35.339×10^6	168.67	<0.0001
Rearing Technique (R)	1	21.792×10^6	104.01	<0.0001
A × R	2	10.137×10^6	48.39	<0.0001
Error	12	2.514×10^6		
Cholesterol				
Age (A)	2	51.061×10^3	63.74	<0.0001
Rearing Technique (R)	1	161.772×10^3	201.95	<0.0001
A × R	2	47.464×10^3	59.25	<0.0001
Error	12	0.801×10^3		
Glycogen				
Age (A)	2	1.644	22.69	<0.0001
Rearing Technique (R)	1	0.792	10.93	0.0063
A × R	2	0.360	4.97	0.0267
Error	12	0.870		
Protein				
Age (A)	2	0.016	105.08	<0.0001
Rearing Technique (%)	1	0.064	403.01	<0.0001
A × R	2	0.017	105.90	<0.0001
Error	12	0.002		

juveniles still possessed significantly more triglycerides than *in vitro*-reared animals (Fig. 3).

Cholesterol levels changed significantly during the early development of fish-reared individuals of *Utterbackia imbecillis* (Table 1). Animals at 1 day post metamorphosis had significantly higher amounts of whole-animal cholesterol than did the glochidia (Fig. 4); however, 7-day-old juveniles exhibited a significant decrease. For those animals reared *in vitro*, there was no significant change in the concentration of cholesterol from glochidia to 7-day-old juveniles. Fish-reared juveniles possessed significantly more cholesterol at 1 day and 7 days following metamorphosis than did *in vitro*-reared juveniles.

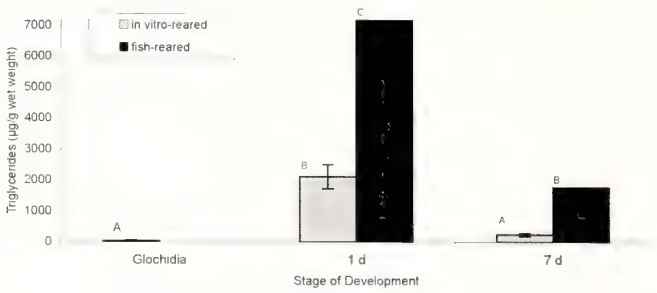

Figure 3. The change in triglyceride concentration in the early life history stages of *Utterbackia imbecillis.* White = glochidia, gray = *in vitro*-reared, black = fish-reared.

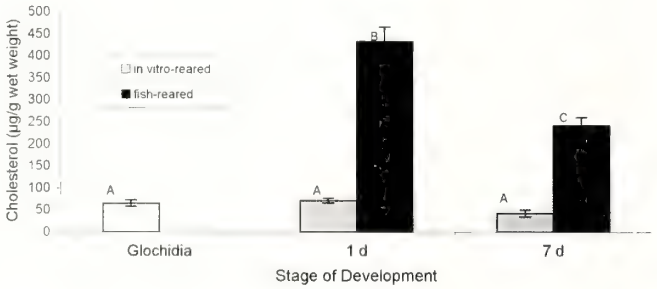

Figure 4. The change in cholesterol levels in the early life history stages of *Utterbackia imbecillis.* White = glochidia, gray = *in vitro*-reared, black = fish-reared. Bars with the same letter are not significantly different ($p < 0.05$)

Glycogen

The concentration of glycogen in glochidia, 1-day-old, and 7-day-old juveniles showed a pattern similar to that found for cholesterol. There was no significant change in the amount of glycogen in the *in vitro*-reared animals at these early stages of development (Fig. 5, Table 1), but among the fish-reared animals, there was a significantly higher amount of glycogen in 1-day-old animals than in the glochidia. In addition, the level of glycogen in 1-day-old fish-reared juveniles was significantly higher than in those reared *in vitro.* However, by the first week following metamorphosis, the glycogen concentration in the fish-reared juveniles had declined to the levels found in the glochidia and in all *in vitro*-reared juveniles.

Protein

The concentration of protein differed significantly among the various age classes and between the two rearing techniques (Table 1). There were no significant changes in

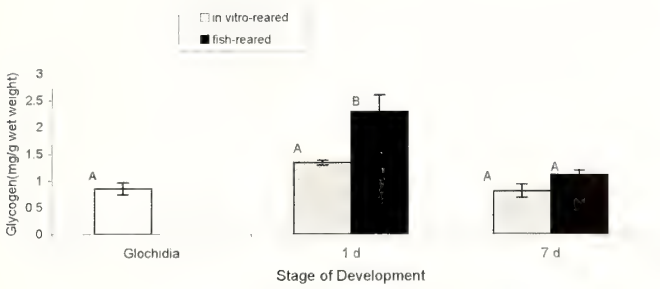

Figure 5. The change in glycogen concentration in the early life history stages of *Utterbackia imbecillis*. White = glochidia, gray = *in vitro*-reared, black = fish-reared. Bars with the same letter are not significantly different ($p < 0.05$).

protein concentrations among *in vitro*-reared animals, but for the fish-reared animals, there was a significantly higher amount of protein in 1-day-old animals compared to the glochidia (Fig. 6) and also in comparison to that of juveniles reared *in vitro*. However, by the first week following metamorphosis, the protein concentration in the fish-reared juveniles had declined to the levels found in the glochidia.

DISCUSSION

There are no widely accepted methods for measuring the physiological condition of the early life history stages of freshwater mussels. Glochidia are commonly assessed for viability by exposing them to a saline solution and monitoring the number that close in response (Huebner and Pynnönen 1992, Goudreau *et al.* 1993, Hansten *et al.* 1996). Fisher and Dimock (2000) examined the correlation of this measure of viability with metamorphosis of larvae and found that closure in response to a salt solution provides an adequate estimation of developmental competence.

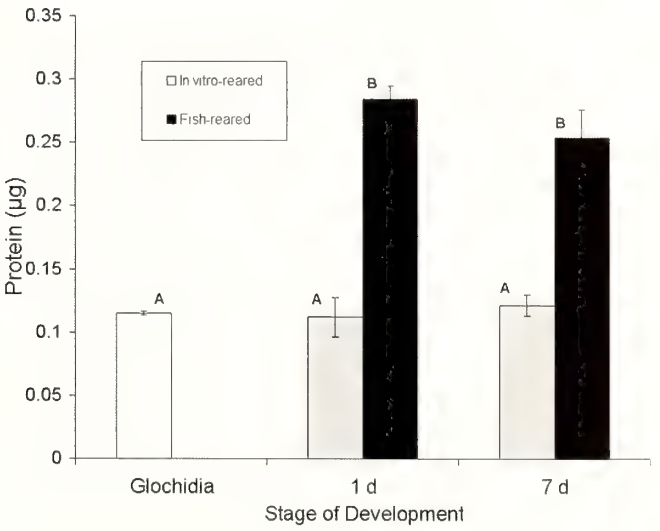

Figure 6. The change in protein concentration in the early life history stages of *Utterbackia imbecillis*. White = glochidia, gray = *in vitro*-reared, black = fish-reared. Bars with the same letter are not significantly different ($p < 0.05$).

Some authors (Gatenby *et al.* 1996, 1997) have used growth and survival to determine the effects of experimental diet on the general health of juvenile mussels or have examined the resistance of juveniles to prolonged exposure to potentially lethal conditions (Dimock and Wright 1993, Fisher 2001). The present study found that growth and survival of juvenile individuals of *Utterbackia imbecillis* were significantly different during the first 14 days following metamorphosis, with fish-reared juveniles growing larger and having significantly greater survivorship. However, these measures are inappropriate for assessing the short-term effects of environmental stress (Naimo and Monroe 1999) or for providing a relatively rapid appraisal of physiological condition.

The results of the biochemical analyses suggest that several parameters of overall metabolism may be useful indicators of the physiological status of juvenile mussels. Fish-reared individuals of *Utterbackia imbecillis* had significantly more triglycerides, cholesterol, glycogen, and protein at 1 day post metamorphosis than did their *in vitro*-reared counterparts. The relatively low levels of cholesterol, glycogen, and protein among *in vitro*-reared animals remained constant between the glochidial stage and 7 days post metamorphosis; however, triglycerides had increased significantly by day 1 following metamorphosis. In contrast, all three compounds were present in significantly greater concentrations in 1-day-old fish-reared juveniles than in glochidia.

Lipids are the principal energy reserve of marine bivalve larvae (Gallager *et al.* 1986) and have been shown to decrease following starvation in juveniles of *Utterbackia imbecillis* (Tankersley 2000). The demonstration that the accumulation of lipids in juveniles of *U. imbecillis* can be influenced by the addition of fish oils to *in vitro* culture media (Tankersley 2000) further underscores the potential importance of this dietary component. Glycogen is an important carbohydrate store and has been used to assess physiological condition in adult unionid bivalves (Haag *et al.* 1993, Naimo and Monroe 1999). The present study indicates that it is a useful measure for larvae and juveniles as well. Protein content has also been used to measure physiological condition in adult bivalves (Mann and Gallager 1985, Gabbot and Peek 1991) and appears to be a relatively sensitive measure of condition for the early life history stages also.

The data presented herein indicate that a number of biochemical parameters, including triglyceride, cholesterol,

glycogen, and protein levels may be used to assess the physiological conditions of larval and juvenile *Utterbackia imbecillis*. However, the large numbers of individuals required for these tests may limit these analyses to mussel species that are easily reared in a laboratory setting until more sensitive assays are available. The consistently higher concentrations of these substances among fish-reared juveniles as compared to animals reared *in vitro* suggest that juvenile mussels reared from glochidia that have encysted on their host fish begin their post-metamorphic life with greater nutrient reserves and are therefore more resistant to thermal and other stresses (Fisher 2001) than juveniles from *in vitro* culturing. Fish-reared juveniles also grow more quickly and have higher initial survival rates than their *in vitro*-reared counterparts. If these observations prove applicable to other unionid species as well, then the use of *in vitro* techniques to rear young mussels for experimental studies or for propagation and reintroduction may warrant further evaluation.

LITERATURE CITED

Bayne, B. L., A. Bubal, P. A. Gabbott, D. R. Livingstone, D. M. Lowe, and M. N. Moore. 1982. Glycogen utilisation and gametogenesis in *Mytilus edulis* L. *Marine Biology Letters* **3**: 89-105.

Bligh, E. G. and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **37**: 911-917.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein dye binding. *Analytical Biochemistry* **72**: 248-254.

Dimock, R. V., Jr. 2000. Oxygen consumption by juvenile *Pyganodon cataracta* in declining PO_2 (Bivalvia: Unionidae). *In:* R. A. Tankersley, D. Warmolts, G. T. Watters, and B. Armitage, eds., *Freshwater Mollusk Symposium Proceedings—Part I: Proceedings of the Conservation, Captive Care, and Propagation of Freshwater Mussels Symposium, Columbus, Ohio, March 1998*. Ohio Biological Survey, Columbus, Ohio. Pp. 1-8.

Dimock, R. V., Jr. and A. H. Wright. 1993. Sensitivity of juvenile freshwater mussels to hypoxic, thermal and acid stress. *Journal of the Elisha Mitchell Scientific Society* **109**: 183-192.

Fisher, G. R. 2001. *Morphology and Physiology of Larval and Juvenile* Utterbackia imbecillis *(Bivalvia: Unionidae)*. Ph.D. Dissertation, Wake Forest University, North Carolina.

Fisher, G. R. and R. V. Dimock, Jr. 2000. Viability of glochidia of *Utterbackia imbecillis* following their removal from the parental mussel. *In:* P. D. Johnson and R. S. Butler, eds., *Freshwater Mollusk Symposium Proceedings—Part II: Proceedings of the First Symposium of the Freshwater Mollusk Conservation Society, Chattanooga, Tennessee, March 1999*. Ohio Biological Survey, Columbus, Ohio. Pp. 185-188.

Fraser, A. J. 1989. Triacylglycerol content as a condition index for fish, bivalve, and crustacean larvae. *Canadian Journal of Fisheries and Aquatic Sciences* **46**: 1868-1873.

Gabbott, P. A. and K. Peek. 1991. Cellular biochemistry of the mantle tissue of the mussel *Mytilus edulis* L. *Aquaculture* **94**: 165-176.

Gallager, S. M. and R. Mann. 1986. Individual variability in lipid content of bivalve larvae quantified histochemically by absorption photometry. *Journal of Plankton Research* **8**: 927-937.

Gallager, S. M., R. Mann, and G. C. Sasaki. 1986. Lipid as an index of growth and viability in three species of bivalve larvae. *Aquaculture* **56**: 81-103.

Gatenby, C. M., R. J. Neves, and B. C. Parker. 1996. Influence of sediment and algal food on cultured juvenile freshwater mussels. *Journal of the North American Benthological Society* **15**: 597-609.

Gatenby, C. M., B. C. Parker, and R. J. Neves. 1997. Growth and survival of juvenile rainbow mussels, *Villosa iris* (Lea, 1829) (Bivalvia: Unionidae), reared on algal diets and sediment. *American Malacological Bulletin* **14**: 57-66.

Goudreau, S. E., R. J. Neves, and R. J. Sheenan. 1993. Effects of wastewater treatment plant effluents on freshwater mollusks in the upper Clinch River, Virginia, USA. *Hydrobiologia* **252**: 211-230.

Grant, J. and B. Thorpe. 1991. Effects of suspended sediment on growth, respiration, and excretion of the soft-shell clam (*Mya arenaria*). *Canadian Journal of Fisheries and Aquatic Sciences* **48**: 1285-1292.

Haag, W. R., D. J. Berg, D. W. Garton, and J. L. Farris. 1993. Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (*Dreissena polymorpha*) in western Lake Erie. *Canadian Journal of Fisheries and Aquatic Sciences* **50**: 13-19.

Hansten, C., M. Heino, and K. Pynnönen. 1996. Viability of glochidia of *Anodonta anatina* (Unionidae) exposed to selected metals and chelating agents. *Aquatic Toxicology* **34**: 1-12.

Huebner, J. D. and K. S. Pynnönen. 1992. Viability of glochidia of two species of *Anodonta* exposed to low pH and selected metals. *Canadian Journal of Zoology* **70**: 2348-2355.

Isom, B. G. and R. G. Hudson. 1982. In vitro culture of parasitic freshwater mussel glochidia. *Nautilus* **96**: 147-151.

Kat, P. W. 1984. Parasitism and the Unionacea (Bivalvia). *Biological Reviews* **59**: 189-207.

Keller, A. E. and S. G. Zam. 1991. The acute toxicity of selected metals to the freshwater mussel, *Anodonta imbecillis*. *Environmental Toxicology and Chemistry* **10**: 539-546.

Kleinman, S., B. G. Hatcher, and R. E. Scheibling. 1996. Growth and content of energy reserves in juvenile sea scallops, *Placopecten magellanicus*, as a function of swimming frequency and water temperature in the laboratory. *Marine Biology* **124**: 629-635.

Lewis, P. A., D. J. Klemm, J. M. Lazorchak, T. J. Norberg-King, W. H. Peltier, and M. Heber. 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms. EPA/600/4-91/002. Environmental Protection Agency, Cincinnati, Ohio.

Lochmann, S. E., G. L. Maillet, K. T. Frank, and C. T. Taggart. 1995. Lipid class composition as a measure of nutritional condition in individual larval Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences* **52**: 1294-1306.

Mann, R. and S. M. Gallager. 1985. Physiological and biochemical energetics of larvae of *Teredo navalis* L. and *Bankia gouldi* (Bartsch) (Bivalvia: Teredinidae). *Journal of Experimental Marine Biology and Ecology* **85**: 211-228.

McKinney, A. D. and D. C. Wade. 1996. Comparative response of *Ceriodaphnia dubia* and juvenile *Anodonta imbecillis* to pulp and paper mill effluents discharged to the Tennessee River and its tributaries. *Environmental Toxicology and Chemistry* **15**: 514-517.

Naimo, T. J. and E. M. Monroe. 1999. Variation in glycogen concentrations within mantle and foot tissue in *Amblema plicata plicata*: Implications for tissue biopsy sampling. *American Malacological Bulletin* **15**: 51-56.

Naimo, T. J., E. D. Damschen, R. G. Rada, and E. M. Monroe. 1998. Nonlethal evaluation of the physiological health of unionid mussels: Methods for biopsy and glycogen analysis. *Journal of the North American Benthological Society* **17**: 121-128.

Ouellet, P., C. T. Taggert, and K. T. Frank. 1992. Lipid condition and survival in shrimp (*Pandalus borealis*) larvae. *Canadian Journal of Fisheries and Aquatic Sciences* **49**: 368-378.

Palacios, E., C. I. Pérez-Rostro, J. L. Ramírez, A. M. Ibarra, and I. S. Racotta. 1999. Reproductive exhaustion in shrimp (*Penaeus vannamei*) reflected in larval biochemical composition, survival and growth. *Aquaculture* **171**: 309-321.

Patterson, M. A., B. C. Parker, and R. J. Neves. 1999. Glycogen concentration in the mantle tissue of freshwater mussels (Bivalvia: Unionidae) during starvation and controlled feeding. *American Malacological Bulletin* **15**: 47-50.

Polhill, J. B. and R. V. Dimock, Jr. 1996. Effects of temperature and pO_2 on the heart rate of juvenile and adult freshwater mussels (Bivalvia: Unionidae). *Comparative Biochemistry and Physiology* **113A**: 1-7.

Tankersley, R. A. 2000. Fluorescence techniques for evaluating the lipid content of larval and juvenile freshwater mussels. *In:* A. Tankersley, D. Warmolts, G. T. Watters, and B. Armitage eds., *Freshwater Mollusk Symposium Proceedings—Part I: Proceedings of the Conservation, Captive Care, and Propagation of Freshwater Mussels Symposium, Columbus, Ohio, March 1998.* Ohio Biological Survey, Columbus, Ohio. Pp. 115-126.

Waller, D. L. and L. G. Mitchell. 1989. Gill tissue reactions in walleye *Stizostedion vitreum vitreum* and common carp *Cyprinus carpio* to glochidia of the freshwater mussel *Lampsilis radiata siliquoidea*. *Diseases of Aquatic Organisms* **6**: 81-87.

Warren, L. W., S. J. Klaine, and M. T. Finley. 1995. Development of a field bioassay with juvenile mussels. *Journal of the North American Benthological Society* **14**: 341-346.

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Freshwater Molluscs of Fort Stewart, Georgia, U.S.A.

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Abstract: During the summers of 2001 and 2002, 94 sites were surveyed for unionid mussels on the Fort Stewart U.S. Army Installation in Georgia. Ft. Stewart is drained by the Canoochee and Ogeechee rivers and is located in the Lower Coastal Plain of Georgia. The purpose of this study was to survey all aquatic habitats on Fort Stewart Military Installation, so that species composition and distribution of any existing mussel populations could be determined. Eleven species of native freshwater unionids were identified. One additional unionid species, *Elliptio shepardiana* was observed at a location adjacent to the Fort Stewart boundary on Beards Creek, a second-order headwater stream of the Altamaha drainage. The Asian clam *Corbicula fluminea* and eight species of freshwater gastropods were also observed. The Asian clam was observed at all sample sites except in Beards Creek. High unionid abundance was limited to three survey locations in 2001. The first site was a series of old abandoned borrow pits within the floodplain of the Canoochee River with an abundance of 10.5 mussels/person-hour (no. hr^{-1}); the other two sites were below the Hinesville/Fort Stewart Regional Water Pollution Control Plant (WPCP) on Taylors Creek at bridge 29 with a mussel abundance of 260 no. hr^{-1} and Canoochee Creek at bridge 27 with an abundance of 310 no. hr^{-1}. In 2002, highest mussel abundance was limited to one site at the confluence of Taylors and Canoochee creeks upstream of the WPCP with a mussel abundance of 192 no. hr^{-1}. The abundances and species richness of mussels correlated with alkalinity and pH. Concentrations of total phosphorus up to 6 mg/L appeared to favor mussel abundance.

Key words: Mussels, bivalves, coastal plain, blackwater, unionid, gastropod, Sphaeriidae

Two types of rivers are prevalent in the Georgia Coastal Plain: (1) rivers that originate in the Piedmont and carry loads of red clay and (2) the blackwater rivers that originate on the Coastal Plain. Blackwater rivers carry little sediment and are colored brown to black by suspended organic matter (Boyd 1976). Blackwater streams occur at relatively low elevations. They have warm water in summer (between 18.0 and 26.0°C), quiet flows, high turbidities, and more pools and fewer riffles than coldwater rivers existing in more northerly latitudes. Substrates of small particle size, rooted and floating vegetation, and sparse shade and cover typify blackwater rivers (Winger 1981).

Schmitt (1988) refers to the Canoochee River as a non-alluvial blackwater system in which tannins are released from tree roots and decaying vegetation from adjacent riverine swamp areas. The Canoochee River is the largest tributary of the Ogeechee River, spans 164.5 km, and is a fifth-order woodland stream (C. Canolas, pers. comm.). Allochthonous organic matter predominates along with considerable woody debris in the Canoochee River (Lewis *et al.* 1981). This system drains low pH/alkalinity Pleistocene sands and clays of the Coastal Plain. These substrates are important streambed features that affect the distribution of fish and benthic invertebrates in the Canoochee (Lewis and Tutora 1998). The Canoochee is considered to be in the mixed land-use class due to its passage through many different land-use types (Lewis and Tutora 1998). Silviculture predominates, followed by old crop fields and agricultural pastureland. Urbanized sprawl constitutes 10-30% of the remaining land in the basin, along with scattered wetlands (Lewis and Tutora 1998). The Canoochee system has its headwaters northeast of Swainsboro, Emanuel County, Georgia, and flows southeast to the Atlantic Ocean (Boyd 1976). The total area of this watershed is 364,797 ha (C. Canolas, pers. comm.).

Riverine ecosystems may contain the highest diversity of freshwater molluscs due to their relative permanence and contain a greater heterogeneity of physio-chemical characteristics and biological niches for aquatic organisms. Small headwater streams with swift current and allochthonous energy contributions, as well as large Coastal Plain rivers with slow flow and allochthonous production, provide needed habitat for molluscan fauna (Neves *et al.* 1997). The current distributions of freshwater molluscan species have been influenced by natural and anthropogenic factors. According to Stites *et al.* (1995), some of these natural factors include pH, alkalinity concentration within the water column, and seston composition. However, differentiating between these factors

is difficult without sufficient historic surveys and collection records to support conclusions (Neves *et al.* 1997). Populations of freshwater mussels in the family Unionidae were once major parts of the southeastern aquatic faunal diversity, but are now in decline and fast disappearing (Keferl 1993). The most threatened freshwater organisms in the eastern United States belong to this family (Neves 1991).

Because stream environments are highly susceptible to human disturbances (Gillies *et al.* 2003), and habitat destruction is a primary reason for mussel species loss in many southeastern streams (Williams *et al.* 1993), we wanted to investigate possible bivalve populations in all Fort Stewart waters. The purpose of this study was to survey all swamps, creeks, streams, rivers, ponds, and lakes on the Installation so that species composition and distribution of mussel populations could be determined. The Ogeechee River forms the eastern boundary of Fort Stewart and the Canoochee River flows in a southeasterly direction through the installation (Fig. 1). Beards Creek drains the western boundary of Fort Stewart.

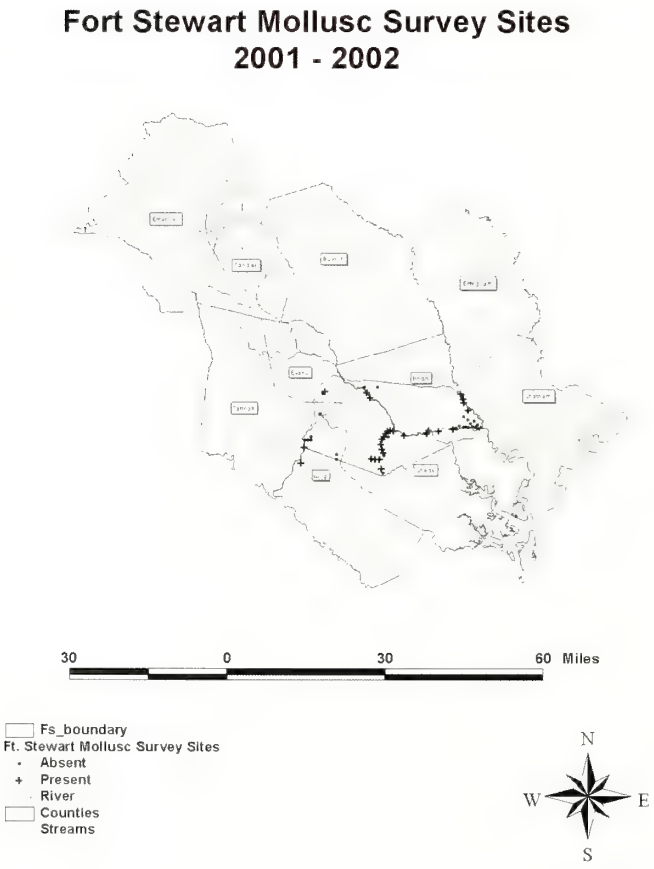

Figure 1. Overview of river drainage and sampling area. Fort Stewart is in the shaded area. Sites where bivalves are present are represented by a + and sites where bivalves are absent are represented by a ●.

Prior to this survey, nothing was known of the unionid fauna on the installation. Apparently, the activities at Fort Stewart Military Installation have not altered the natural flow of the Canoochee River since the establishment of the installation in 1940. The floodplain of the Canoochee River is extensive and currently is well protected. However, the waters flowing through the installation originate outside Fort Stewart and are subject to point and non-point pollution sources typical of the drainages of coastal Georgia.

The Ogeechee River has a diverse mussel fauna (Johnson 1970, Alderman 1991), but has never been surveyed systematically. Alderman (1991) surveyed a small part of the Ogeechee River for the Atlantic pigtoe, *Fusconaia masoni* (Conrad, 1834), a critically imperiled mussel in Georgia and North Carolina. Fuller (1973) also reported the Atlantic pigtoe from the Ogeechee River system, but did not report any other species. Johnson (1970) published a summary of all the Unionidae in the entire southern Atlantic Slope region from Virginia to Florida. He summarized what was known about the distribution of mussels in the Ogeechee River system from museum records. According to the historical records, 15 unionid species have been reported from the Ogeechee River system (Table 1).

METHODS

Unionid survey

Systematic surveys of the Canoochee River and its tributaries, along with several off-post sites, were completed during July and August in 2001 and April through September 2002 (31 sites in 2001 and 63 sites in 2002). Abundances and diversity of Fort Stewart mussels were determined and compiled into a database for the entire Ogeechee River system. Ninety-four sites were selected from the accessible waters on Fort Stewart. Habitats surveyed included swamps, ponds, lakes, small streams, the Ogeechee River, and the Canoochee River. The extent to which each area was sampled and the amount of time spent examining each site varied depending upon the substrate, amount of particulate organic matter present at the site, and hydrological features such as stream meander, presence of riffles, or stream straight flow.

Investigators chose a two-stage sampling regime as suggested by Strayer and Smith (2003). The premise is based upon large-scale population estimates where population estimate is determined by how many sites are sampled rather than how many quadrats are sampled within a site. Based on the number of person-hours, the catch per unit effort (CPUE) time was determined. The size of the area searched

Table 1. Unionidae recorded from the Ogeechee River System.

Species	Common name	Historical[1]	2001	2002
Subfamily Unioninae				
Fusconaia masoni (Conrad, 1834)	Atlantic pigtoe	X		
Elliptio angustata (I. Lea, 1831)	Carolina lance	X		X
Elliptio complanata (Lightfoot, 1786)	Eastern elliptio	X		
Elliptio complanata form *conferta* (I. Lea, 1834)	Unnamed			X
Elliptio congaraea (I. Lea, 1831)	Carolina slabshell	X	X	X
Elliptio congaraea form *corvus* (I. Lea, 1859)	Unnamed			X
Elliptio folliculata (I. Lea, 1838)	Pod lance	X		
Elliptio icterina (Conrad, 1834)	Variable spike	X	X	X
Elliptio lugubris (I. Lea, 1834)	Sad elliptio		X	X
Elliptio producta (Conrad, 1836)	Atlantic spike	X		
Uniomerus carolinianus (Bosc, 1801)	Florida pondhorn	X	X	X
Subfamily Antodontinae				
Alasmidonta undulata (Say, 1817)	Triangle floater	X		
Anodonta couperiana I. Lea, 1840	Barrel floater		X	X
Pyganodon cataracta (Say, 1817)	Eastern floater	X	X	X
Utterbackia imbecillis Say, 1829	Paper pond shell	X	X	X
Subfamily Lampsilinae				
Villosa delumbis (Conrad, 1834)	Eastern creekshell	X	X	X
Villosa vibex (Conrad, 1834)	Southern rainbow	X		
Lampsilis cariosa (Say, 1817)	Yellow lamp mussel	X		
Lampsilis splendida (I. Lea, 1838)	Rayed pink fatmucket	X		

[1] Records modified from Johnson (1970) and Alderman (1991).

depended upon the type of habitat present and the likelihood of finding freshwater mussels. Most of the sampling effort was focused on those sites characterized by variable unconsolidated sediments such as silts, muds, sands, and gravels. Sites with little water or sites with extensive hardpan clay substrate did not warrant as much search time.

Sampling methods included visually searching in shallow water for mussel siphons, searching for the remains of old shells, and hand picking or "noodling" for mussels in the sediments of waters <1 m deep. In deeper waters, modified clam and garden rakes were used to examine the substrate for mussels. Because most mussels were found in unconsolidated sediments in protected areas near the banks, greater noodling effort was conducted in these locations. No attempt was made to SCUBA dive or snorkel in lacustrine or fluvial sites due to low visibility. When water levels were low, searchers waded into these sites searching for molluscs by feeling and raking the substrate.

When mussels were found, they were placed in a mesh bag and kept in the water. After sampling was complete, all mussels were identified, counted, and most were released. Those kept were saved and will be used as voucher specimens to be given to the Georgia Museum of Natural History. Members of *Elliptio* spp. were identified using the keys and species lists of Davis and Mulvey (1993), Watters (2001), and Turgeon *et al.* (1998). We also used collected specimens to confirm previous identifications within the genus *Elliptio*.

In areas where three or more species of mussels were found, restricted timed searches were made. In restricted searches, all samplers searched a defined stream or pond section only in the area where mussel beds were found. The restricted searches were set between 15 and 120 minutes depending upon numbers of mussels present at each site. Three restricted timed searches were conducted in 2001 and five timed searches were conducted in 2002 below the Hinesville/Fort Stewart Regional Water Pollution Control Plant (WPCP) effluent canal (Fig. 2).

Measurements of water quality

Alkalinity was measured using the bromcresol indicator/1.8 N sulfuric acid titration procedure provided in the HACH FF2-Fish Farming Kit. The hand-held titrator automatically calibrated when set to zero. Ambient water temperature, specific conductance, dissolved oxygen, total dissolved solids, pH, and turbidity were measured at each site using a Hydrolab® Quanta. Probes on the Quanta were inserted directly into the stream flow. The Quanta unit was calibrated every month. Total dissolved phosphorous (TDP), total inorganic nitrogen (TIN), nitrate nitrogen (NO_3-N), and copper (Cu) metal concentrations of grab samples taken

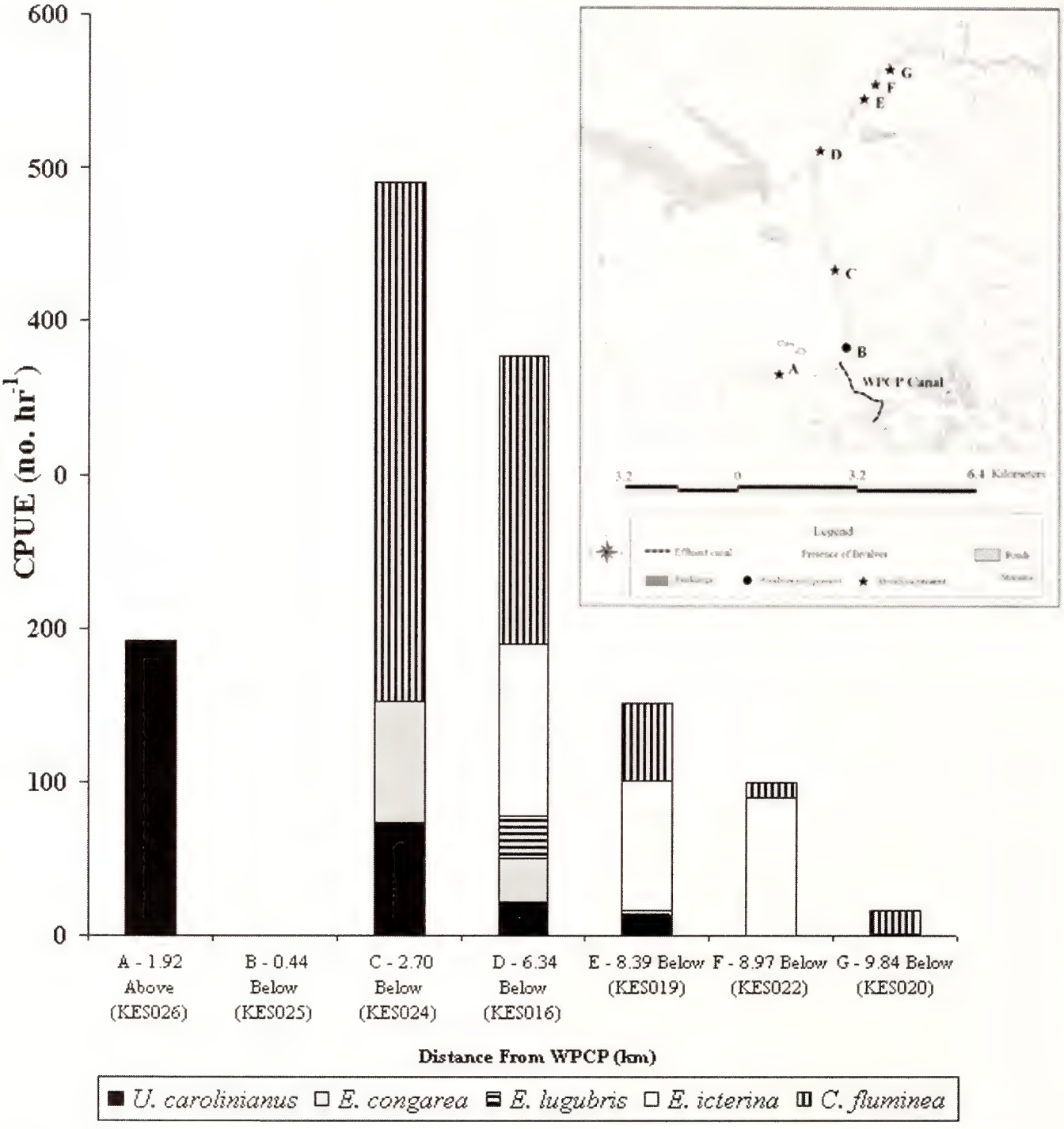

Figure 2. Fort Stewart Mollusc Survey Sites on Taylors and Canoochee creeks during the 2002 sampling season.

at field sites were measured using a HACH DR4000 Spectrophotometer. All analytical procedures were executed using methods approved by the Environmental Protection Agency as prescribed by HACH.

RESULTS

Survey

Molluscan diversity in the Canoochee River is low. In 2001, eight living mussel species were found at 10 different

sites. Three more mussel species were identified in 2002, yielding a total of 11. Eight of these were found in previous collections (Table 1). A total of 37.42 person-hours was spent in 2001 and 61.85 person-hours were spent in 2002. The mean number of individuals collected per hour at sites where mussels were found in 2001 was 145.5 ± 73.3. The mean number of mussel species collected per hour at sites where mussels were found in 2001 was 2.5 ± 1.1, and the mean number of molluscan species collected per hour where molluscs were found in 2001 was 3.1 ± 2.5. The average CPUE for individuals collected for all sites was 38.8 no. hr^{-1} in 2001.

The average number of individuals collected per hour where mussels were found in 2002 was 80.0 ± 73.0 and average numbers of mussel species collected per hour where mussels were found in 2002 was 3.0 ± 2.1. The average number of molluscan species collected per hour where molluscs were found in 2002 was 2.8 ± 1.8. The average CPUE for individuals collected for all sites was 32.0 no. hr^{-1} in 2002. Two of the three new unionid species collected in 2002 were living: *Elliptio complanata* form *conferta* (I. Lea, 1834) and *Elliptio angustata* (I. Lea, 1859); *Elliptio congaraea* form *corvus* (I. Lea, 1859) was the species found in 2002 but not in 2001 for which no living specimens were found. The greater eastern peaclam *Pisidium dubium* (Say, 1817) from the family Sphaeridae was identified. No detailed record of the density of the exotic *Corbicula fluminea* (Muller, 1774) was made. Its presence was noted and the frequency of occurrence was estimated.

In 2001, the three most productive areas for freshwater mussels in terms of CPUE were the B-8 borrow ponds on the flood plain of the Canoochee River, site EPK007 (10.5 no. hr^{-1}); Canoochee Creek-bridge 27, site EPK016 (310 no. hr^{-1}); and Taylors Creek-bridge 29, site EPK018 (260 no. hr^{-1}). Seven of the 8 mussel species found in 2001 were found at these three sites. Only *Elliptio congaraea* (I. Lea, 1831) was found elsewhere. Five mussel species were found at the B-8 borrow ponds, site EPK007 (Table 2). However, mussel abundance was highest at Canoochee and Taylors creeks. *Elliptio lugubris* (I. Lea, 1834) comprised 73.5% of

Table 2. Timed searches with highest mussel abundances on Taylors and Canoochee Creeks at eight survey sites below the Water Pollution Control Plant (WPCP) effluent canal for 2001-2002.

Location	EPK 007 2001 Borrow ponds	EPK 016 2001 Bridge 27	EPK 018 2001 Bridge 29	KES 026 2002 Above WPCP	KES 024 2002 Bridge 29	KES 016 2002 Bridge 27	KES 019 2002 Bridge 27	KES 022 2002 Bridge 29
Anodonta couperiana	1 (2.4)*	0	0	0	0	0	0	0
Elliptio icterina	0	21 (13.5)	14 (10.8)	0	0	448 (59.2)	128 (83.1)	45 (100)
Elliptio congaraea	0	0	0	0	88 (52.4)	115 (15.2)	0	0
Elliptio lugubris	0	114 (73.5)	86 (66.2)	0	0	107 (14.2)	4 (2.6)	0
Elliptio congaraea form *corvus*	0	0	0	0	0	0	3 (2.0)	0
Uniomerus carolinianus	33 (78.6)	20 (12.9)	30 (23.0)	48 (100)	80 (47.6)	86 (11.4)	19 (12.3)	0
Pyganodon cataracta	1 (2.4)	0	0	0	0	0	0	0
Utterbackia imbecillis	6 (14.2)	0	0	0	0	0	0	0
Villosa delumbis	1 (2.4)	0	0	0	0	0	0	0
Corbicula fluminea†	0	0	0	0	371	746	75	5
Total count	42	155	130	48	168	756	154	45
CPUE (no. hr^{-1})	10.5	310	260	192	152.7	189	102	90

* Numbers in parentheses are percentages of catch at each site.

† Not in counts.

individuals collected at Canoochee Creek-bridge 27, site EPK016 (Table 2). The Taylors Creek bridge 29, site EPK018, contained the second highest abundances in 2001 for *Elliptio icterina* (Conrad, 1834) and *E. lugubris* reaching 10.8% and 66.2% of the total number of individuals collected for each species, respectively (Table 2).

In 2002, site KES026 at the confluence of Taylors and Mill creeks upstream of the WPCP effluent canal had the highest CPUE of 192 no. hr^{-1} and only *Uniomerus carolinianus* (Bosc, 1801) was collected. Downstream of the WPCP, abundance was highest in Canoochee Creek at sites KES016, KES019, and KES022, and Taylors Creek site KES024 (Table 2). These sites had the highest CPUE of all surveyed sites. Site KES016 on Canoochee Creek had the highest CPUE (189.0 no. hr^{-1} and 756 individuals) downstream of the WPCP at bridge 27. Four species were present at Fort Stewart site KES016, including 448 individuals of *Elliptio icterina* (59.2% of total catch); 115 individuals of *Elliptio congaraea* (15.2% of total catch), which is currently being tracked as a species of special concern by the Georgia Natural Heritage Program (B. Albanese, personal communication); 107 *Elliptio lugubris* (14.2% of total catch); and 86 *U. carolinianus* (11.4% of the total catch) (Table 2). Site KES024 on Taylors Creek downstream of the WPCP had the second highest CPUE of 152.7 no. hr^{-1}. At site KES024, 168 individuals were collected where 52.4% of the total catch (88) was *E. congaraea* and 47.6% of the total catch (80) was *U. carolinianus* (Table 2). The third highest CPUE was at site KES019 downstream of the WPCP on Canoochee Creek. At site KES019, 154 individuals were collected (102.0 no. hr^{-1}), where 128 individuals (83.1% of the total catch) were of *E. icterina*, 4 were *E. lugubris* (2.6% of total catch), 3 were *Elliptio congaraea* form *corvus* (2.0% of the total catch), and 19 were *U. carolinianus* (12.3% of total catch). At site KES022, one species of mussel was collected, *E. icterina*, with a CPUE of 90 no. hr^{-1}. No mussels or individuals of *Corbicula fluminea* were found at site KES025, 0.44 km downstream of the WPCP effluent canal, and only *C. fluminea* was found at site KES020, 9.84 km downstream of the WPCP effluent canal (Fig. 2).

Gastropods collected in 2001 were *Physa acuta* (Draparnaud, 1805) and *Campeloma limum* (Anthony, 1860). In 2002, four freshwater snails and two freshwater limpets were found. Specimens of *Goniobasis catenaria postelli* (Say, 1822), *Planorbella trivolvis* (Say, 1817), *Purgulopsis halcyon* (F. G. Thompson, 1977), and *Valvata bicarinata* (Say, 1817) were collected. The limpets were identified to the genera *Levapex* and *Ferrissia*.

Abundances of mussels in the Canoochee and Taylors creeks were high compared to abundances at other sites surveyed. *Elliptio icterina* was the most abundant species in the 2002 survey. Mussel abundance and richness increased with distance downstream from the WPCP (Fig. 2). The only species present above the effluent canal was the native species *Uniomerus carolinianus*, which appeared to be tolerant of a wider range of flows and habitat types than other freshwater bivalves. At the confluence of the effluent canal and Taylors Creek, no mussels were found. However, abundance and richness increased 2.7 km below the effluent canal discharge at site KES024 (Fig. 2). At site KES016, 6.3 km downstream from the effluent canal discharge, abundance decreased but richness increased. Moving farther downstream, abundance as well as richness declined. Only individuals of the exotic *Corbicula fluminea* were present at site KES020 closer to the tannic Canoochee River.

Water Quality

There was a positive linear relationship between alkalinity and CPUE (Fig. 3). As concentrations of calcium carbonate within the water column increased, mussel CPUE increased (R^2 = 0.8421). Alkalinity levels were low in the surrounding swamp areas (3 mg L^{-1}) and on the Canoochee River (17 mg L^{-1}). However, concentrations of calcium carbonate in the Taylors Creek/Canoochee Creek drainage below the WPCP effluent canal were greater than 70 mg L. Sites on the Taylors and Canoochee creeks had an average pH of 7.0. In this study, waters that had higher pH values had greater mussel abundance. CPUE peaked at a pH value of approximately 7.2. Mussel catches increased at pH values above 7.0. No mussels were found in waters below pH 5.9.

The relation that existed between mussel CPUE and the concentration of total dissolved phosphorous (TDP) was unclear. Mussel CPUE tended to increase up to 310 no. hr^{-1} at a total maximum phosphorous concentration of 5.45 mg L^{-1}. Lower mussel CPUE was observed in waters that had TDP concentrations less than 3 mg L^{-1} and greater than 8 mg L^{-1}.

DISCUSSION

Seventy-three percent of historically-noted mussel species in the Ogeechee River system were found on Fort Stewart. Based on their studies of museum voucher specimens, Johnson (1970) and Alderman (1991) identified 15 species of Unionidae that should exist in the Ogeechee River system. However, only 11 species were found in this study. This discrepancy could be due to mis-identification of species or loss of species due to anthropogenic effects.

Mussel abundance and richness increased in two tributaries of the Canoochee River on Taylors Creek and Canoochee Creek downstream of the WPCP effluent canal. In 2001, 260 no. hr^{-1} were collected on Taylors Creek at site EPK018. At site EPK016 on Canoochee Creek, 310 no. hr^{-1}

were collected. Three species of mussels were present at each site.

In 2002, highest mussel abundance upstream from the WPCP was at the confluence of Taylors and Mill creeks, site KES026, with a CPUE of 192 no. hr^{-1} and with one species present, *Uniomerus carolinianus*, which is tolerant of desiccation and low pH. Site KES016 had the highest CPUE downstream of the WPCP effluent canal, with a catch of 189.0 no. hr^{-1}, and four species were present: *Elliptio icterina*, *Elliptio congaraea*, *Elliptio lugubris*, and *U. carolinianus*. Site KES024 on Taylors Creek had the second highest CPUE downstream of the WPCP effluent canal with a catch of 152.7 no. hr^{-1}. *Elliptio congaraea* and *U. carolinianus* were collected at this site. The third highest CPUE downstream of the WPCP effluent canal was at site KES019 with a catch of 102 no.hr^{-1}. Four species were collected at this site: *E. icterina*, *E. lugubris*, *Elliptio congaraea* form *corvus*, and *U. carolinianus* (Fig. 2 and Table 2).

Approximately 0.5 km downstream of the effluent canal no species were present. However, between 2.70 km and 6.34 km downstream of the effluent canal, CPUE and richness increased. The WPCP canal serves as a source for introduced nutrients into the low productive blackwater swamp environment. Because TDP concentration increased in this creek section between Fort Stewart bridges 27 and 29, it is possible that mussel numbers increased due to increased phytoplankton responses from this outside source of TDP. The relation between mussel CPUE and TDP concentration indicated that mussel abundance may be positively correlated to TDP concentrations in the water up to a level beyond which the impacts of eutrophication may negatively influence CPUE. In future studies, TDP concentration needs to be measured in conjunction with mussel abundance.

Results of this study differ from other studies that suggest that introduced effluent degrades mussel habitat. According to Lewis *et al.* (1981), increased sewage effluent negatively modifies natural fish assemblages, which in turn negatively modify mussel assemblages. Neves (1991) found that anthropogenic effects can lead to a decrease in mussel diversity. Abundance and richness were probably higher for the Taylors Creek/Canoochee Creek system because nutrient input from the WPCP effluent provided enrichment to the base of the food web. This added enrichment may have facilitated mussel growth and increased abundance and richness. Blackwater Coastal Plain streams are inherently low nutrient systems, have extremely low pH waters and low alkalinities and dissolved oxygen concentrations (Winger 1981).

Because there appeared to be higher mussel richness and abundance in the Taylors/Canoochee creek system related to the WPCP effluent, the relationship between mussel richness and effluent contributions to ambient water quality conditions needs to be assessed. Concentrations of calcium carbonate were higher in the Taylors/Canoochee creek stream segments compared to other surveyed sites and were as high as 78 mg L^{-1}. Mussel abundance and richness were positively correlated with increasing alkalinity concentrations within the water column (Fig. 3). This is consistent with Downing *et al.* (2000).

In comparison, mussel abundances and species numbers remained low in the Canoochee River as well as in waters of Taylors Creek upstream of the effluent outfall in the tannic swamp. There were only three species present in the Canoochee River, which is approximately 10.0 km downstream of the WPCP effluent outfall: *Elliptio congaraea*, *Elliptio icterina*, and *Villosa delumbis* (Conrad, 1834). Low numbers of species as well as individuals were found in the river. Low numbers were expected due to alkalinities below 17 mg L^{-1} and pH values less than 6.5. Mussel CPUE increased as the pH of the water increased, which was a similar trend to that of alkalinity. However, the relationship between pH and mussel CPUE was not linear, but appeared to be curvilinear.

Because no historical records exist for mussel populations in the Canoochee basin, it is unknown what these populations used to be in this area. It is probable that populations were historically low in terms of species number and individual number due to the low input of nutrients into this system and the natural acidity of its waters. There are a few native species that can survive low-pH waters and ephemeral conditions that occur in the Coastal Plain blackwater swamp, such as *Uniomerus carolinianus* and *Pisidium dubium*. These two species probably existed here before European settlement, based on their ability to withstand the seasonal dry periods that occur in the Coastal Plain swamp.

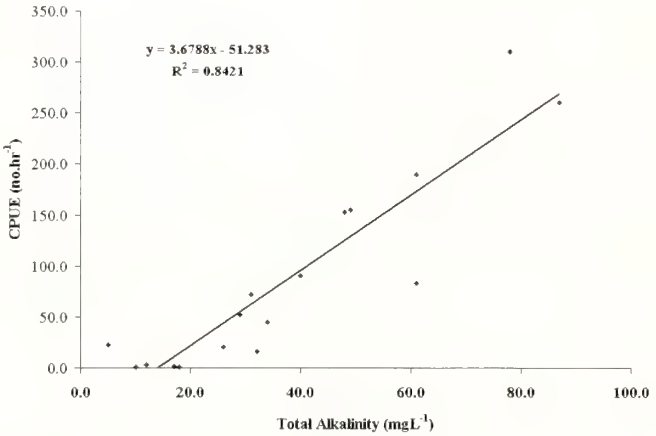

Figure 3. Total alkalinity (mg L^{-1}) regressed upon mussel CPUE (no. hr^{-1}) for the Fort Stewart Mollusc Survey at sites where mussels were present (R^2 = 0.8421).

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LITERATURE CITED

Alderman, J. M., 1991. *Status Survey for the Atlantic Pigtoe (*Fusconaia masoni*) in Georgia*, Nongame Project Report, 1991. Nongame Endangered Wildlife Program, Division of Wildlife Management, North Carolina Wildlife Resources Commission.

Boyd, H. E. 1976. *Biological Productivity in two Georgia River Swamps*. Ph.D. Dissertation, The University of Tennessee, Knoxville.

Davis, G. M. and M. Mulvey. 1993. *Species Status of Mill Creek* Elliptio. Savannah River Site SRO-NERP-22.

Downing, J. A., H. Van Leeuwen, and L. A. DiPaolo. 2000. Substratum patch in the lacustrine mussels *Elliptio complanata* and *Pyganodon grandis*. *Freshwater Biology* **44**: 641-648.

Fuller, S. L. H. 1973. *Fusconaia masoni* (Conrad, 1834) (Bivalvia: Unionacea) in the Atlantic drainage of the Southeastern United States. *Malacological Review* **6**: 105-117.

Gillies, R. R., J. R. Brim Box, J. Symanzik, and E. J. Rodemaker. 2003. Effects of urbanization on the aquatic fauna of the Line Creek watershed, Atlanta - a satellite perspective. *Remote Sensing of Environment* **86**: 411-149.

Johnson, R. I. 1970. The systematics and zoogeography of the Unionidae (Mollusca: Bivalvia) of the Southern Atlantic slope region. *Bulletin of the Museum of Comparative Zoology* **140**: 263-449.

Keferl, E. P. 1993. The status of freshwater mussels in some Georgia, South Carolina, and North Carolina Waters. *In:* K. J. Hatcher, ed., *Proceedings of the 1993 Georgia Water Resources Conference.* University of Georgia Press, Athens, Georgia. Pp. 298-302.

Lewis, L. J. and M. Tutora. 1998. *Stream Habitat Characteristics at Selected Sites in the Georgia-Florida Coastal Plain*. United States Geological Survey Water-resources Investigations Report 98-4013.

Lewis, W. M., R. C. Heidinger, M. H. Paller, and L. J. Wawronowicz. 1981. Effects of municipal sewage on fish communities in selected Illinois streams. *In*: L.A. Krumholtz, ed., *Proceedings of the Warmwater Streams Symposium*. American Fisheries Society, Southern Division, Lawrence, Kansas. Pp. 224-240.

Neves, R. J. 1991. Mollusks. *In*: K. Terwilliger, ed., *Virginia's Endangered Species*. McDonald and Woodward Publishing Co., Blacksburg, Virginia. Pp. 251-320.

Neves, R. J., A. E. Bogan, J. D. Williams, S. A. Ahlstedt, and P. W. Hartfield. 1997. Status of aquatic mollusks in the southeastern United States: A downward spiral of diversity. *In*: G. W. Benz and D. E. Collins, eds., *Aquatic Fauna in Peril: The Southeastern Perspective*. Lenz Design and Communications, Decatur, Georgia. Pp 44-86.

Schmitt, D. N. 1988. *A Fisheries Survey of the Ogeechee River*. Georgia Department of Natural Resources, Game and Fish Division, city of publication.

Stites, D. L., A. C. Benke, and D. M. Gillespie. 1995. Population dynamics, growth, and production of the Asian clam, *Corbicula fluminea* in a blackwater river. *Canadian Journal of Fisheries and Aquatic Sciences* **52**: 425-437.

Strayer, D. L. and D. R. Smith. 2003. A guide to sampling freshwater mussel populations. *American Fisheries Society Monograph* **8**: 10-18.

Turgeon, D. D., J. F. Quinn, Jr., A. E. Bogan, E. V. Coan, F. G. Hochberg, W. G. Lyons, P. M. Mikkelsen, R. J. Neves, C. F. E. Roper, G. Rosenberg, B. Roth, A. Scheltema, F. G. Thompson, M. Vecchione, and J. D. Williams. 1998. *Common and Scientific Names of Aquatic Invertebrates from the United States and Canada: Mollusks*, 2nd Ed. American Fisheries Society special publication 26, Bethesda, Maryland.

Watters, T. G. Electric *Elliptio* Land in North America. Available at: http://www.biosci.ohio-state.edu/~molluscs/Elliptio/index.htm 25 June 2002.

Williams, J. D., M. L. Warren, Jr., K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. *Fisheries* **18**: 6-22.

Winger, P. V. 1981. Physical and chemical characteristics of warmwater: A review. In: L. A. Krumholtz, ed., *Proceedings of the Warmwater Streams Symposium*. American Fisheries Society, Southern Division, Lawrence, Kansas. Pp. 32-44.

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Observations on a cohort of the cut-ribbed ark, *Anadara floridana* (Conrad, 1869), from coastal Georgia

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Abstract: On a sampling trip to obtain broodstock of the potentially commercially important blood (*Anadara ovalis*) and ponderous arks (*Noetia ponderosa*), a large number of the uncommon cut-ribbed ark *Anadara floridana* were found in coastal Georgia. Because little is known about the species, we took the opportunity to investigate the population in terms of its community of associated fauna, size at maturity, and sex ratio. The bivalves were found in clusters attached to the tubes of annelid worms (*Diopatra cuprea*) and to the shells of gastropods (*Busycon carica, Busycotypus canaliculatus*) and other bivalves (*Noetia ponderosa*). Fauna occurring within the clusters in decreasing order of abundance included unidentified barnacles, *Nassarius vibex*, *Nereis succinea*, *Modiolus americanus*, *Musculus lateralis*, *Astyris lunata*, and *Neopanope sayi*. Between 3 and 17 arks occurred on whelk shells and ranged in shell length from 5 to 25 mm. Larger clusters (12 to 56 arks) were observed around the polychaete tubes and included much smaller specimens ranging in length from 0.70 to 28.60 mm. Histological examination of the gonads of 124 arks (shell length range 6.79 to 21.44 mm) revealed a male to female ratio of 1.53:1. All arks examined exhibited gametogenic development and most were in the partially spawned stage, indicating a very small size at first maturity. No hermaphrodites were observed, nor was sexual dimorphism evident in shell length, width, height, or overall wet weight.

Key words: associated fauna, maturity, sex ratio

Many arks worldwide form the basis of economically significant molluscan fisheries and extensive culture operations (Baqueiro *et al.* 1982, Broom 1985, Baqueiro 1989, Manzi and Castagna 1989, Nie 1990, Umezawa 1992). In the United States, ark resources have been largely ignored until recently by the fishing industry. A small fishery, primarily for the blood ark *Anadara ovalis* (Bruguiére, 1789) and the ponderous ark *Noetia ponderosa* (Say, 1822) has developed in Virginia. Meat is sold primarily as an ethnic food in Chicago, New York, Los Angeles, and Washington, D.C., or exported to Mexico (McGraw and Castagna 1994, McGraw *et al.* 1996, 1998). In the southeastern United States, there is a growing demand to diversify culture operations for the hard clam (*Mercenaria mercenaria* [Linné, 1758]). Arks represent an increasingly attractive alternate aquaculture candidate; the University of Georgia Shellfish Research Laboratory is investigating their growth and gametogenesis in coastal Georgia and Florida.

A member of the ark shell family (Arcidae), the cut-ribbed ark *Anadara floridana* (Conrad, 1869) is reported to inhabit shallow waters from North Carolina to Florida, and from Texas to the Greater Antilles (Abbott 1974). This is an uncommon ark species and the largest in US waters, ranging from 6.35 to 12.7 cm in length (Abbott 1974). Little information other than morphology and distribution exists for the species. This paper discusses the first observations of morphology, clustering, associated fauna, and size at sexual maturity for *A. floridana* from Georgia, U.S.A.

MATERIALS AND METHODS

Efforts were expended during March 2003 to collect adult broodstock of ponderous and blood arks off the coast of Georgia to pursue the development of hatchery protocol for both species. A commercial whelk-trawling vessel was asked to participate in the search. On 14 March 2003 large numbers of "ark balls" were found off Sea Island, between the Hampton River (31.216°N, 81.306°W) and Gould's Inlet (31.159°N, 81.366°W) in areas known locally as "the boiler" and "the rocks." In the laboratory these ark balls (Fig. 1A) were identified as clusters of young cut-ribbed arks, *Anadara floridana*, that had attached themselves with byssal threads to tube-building annelid worms, *Diopatra cuprea* (Bosc, 1802); channeled whelks, *Busycotypus canaliculatus* (Linné, 1758); knobbed whelks, *Busycon carica* (Gmelin, 1791); and adult ponderous arks, *Noetia ponderosa*.

Whelks and adult arks carrying clusters were held in a raceway supplied with continuously running seawater at the laboratory on Skidaway Island, and the remaining clusters (around tube worms) were placed in lantern and pearl nets suspended off a dock in the Skidaway River. One week later seven whelks were randomly chosen from the laboratory raceway and their shell lengths (spire apex to siphonal canal) were determined on a measuring board. At this time we noted that the arks were quite mobile; many had moved off the shells of whelks and climbed the walls of the raceway to the surface of the water. All arks that remained associated

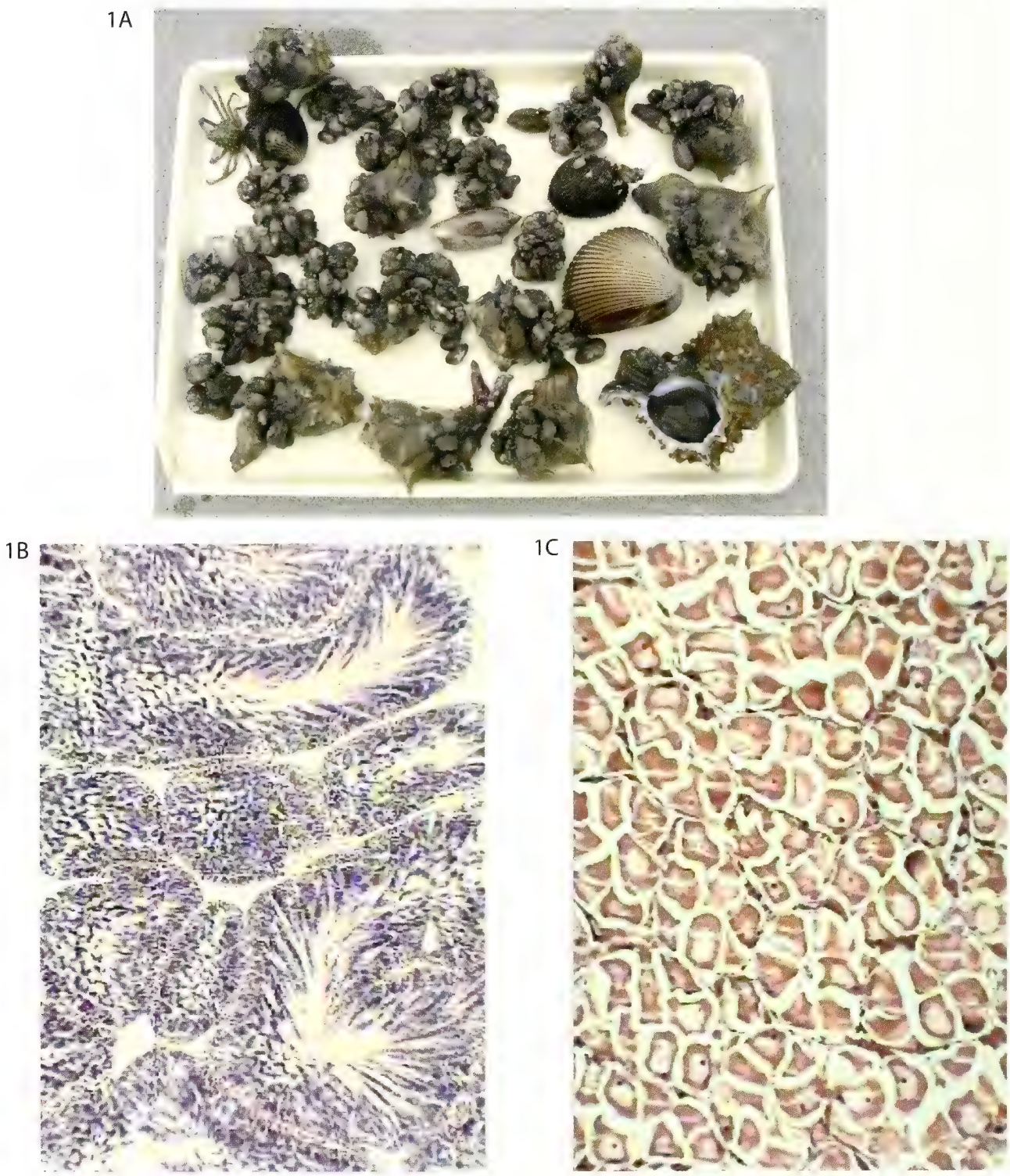

Figure 1. A, Clusters of cut-ribbed arks (*Anadara floridana*) and associated fauna (*Busycon carica, Busycotypus canaliculatus, Dinocardium robustum* [Lightfoot, 1786], *Phyllonotus pomum* [Gmelin, 1791], *Noetia ponderosa, Oliva sayana* [Ravenel, 1834], and *Libinia* sp.). Tray measures 254 mm × 381 mm. B, Histological section of ripe gonadal tissue from a male cut-ribbed ark, *Anadara floridana*. Horizontal field width is 0.62 mm. C, Histological section of ripe gonadal tissue from a female cut-ribbed ark, *Anadara floridana*. Horizontal field width is 0.62 mm.

with the randomly selected whelks were removed, weighed (wet), and shell length, width, and height as defined by McGraw *et al.* (1996) were measured with electronic calipers. The mass of byssal threads was removed from each shell and the material was preserved in 70% ethanol for subsequent sorting and identification. Any barnacles present on the surface of the whelks' shells were also noted. Fifteen clusters of arks from the lantern and pearl nets were randomly selected. The total weight of each cluster was determined. All arks were then separated from each other and measured as described above. Any associated fauna was preserved in 70% ethanol prior to identification.

A total of 124 arks of various sizes were selected from the nets, measured for shell length, width, height (in mm), and wet weight (in g), and the gonads were dissected out for histological analysis. Gonadal tissue was fixed in a 10% buffered formalin solution for 48 hrs, washed with 50% ethanol, and then preserved in 70% ethanol until processing. We processed tissues according to procedures outlined in Howard and Smith (1983). Tissues were sectioned at a thickness of 7 μm and were stained with Harris hematoxylin and counterstained with eosin Y. The examination of prepared gonadal slides was conducted with a Zeiss Standard 20 microscope. Each animal was sexed and assigned to a developmental stage (inactive, early active, late active, ripe, partially spawned, or spent) as described by Walker and Heffernan (1994) and Spruck *et al.* (1994). A linear regression using the reduced major axis method was performed for shell length against width, height, and weight for each sex separately and the lines were compared to investigate possible sexual dimorphism.

RESULTS

The number of arks attached to whelk shells ranged from 3 to 17 (mean ± se = 8.29 ± 1.67), and ranged in shell length from 5.00 to 25.71 mm (18.58 ± 0.65), in width from 2.29 to 20.92 mm (9.30 ± 0.46), in height from 2.73 to 37.59 mm (12.45 ± 0.62), and in wet weight from 0.05 to 3.50 g. In addition to large numbers of barnacle spat (up to 530 on one individual), associated fauna included the predatory ragworm, *Nereis succinea* (Frey and Leuckart, 1847), and the gastropods *Nassarius vibex* (Say, 1822) and *Astyris lunata* (Say, 1826), a scavenger and a predator, respectively. There was no apparent relationship between the numbers of epibionts and the sizes of whelk hosts.

In the clusters attached to the tubes of *Diopatra cuprea* (Bosc, 1802), the number of arks ranged from 12 to 56 (31.40 ± 4.12), while the wet weight of the entire cluster ranged from 15.20 to 44.80 g (26.14 ± 2.55). Individual arks ranged in length from 0.70 to 28.60 mm (10.27 ± 0.98), in width from 0.30 to 14.00 mm (4.98 ± 0.44), in height from 0.20 to 25.00 mm (6.45 ± 0.65), and in wet weight from almost 0 to 4.20 g (0.69 ±0.09). No living polychaete worm was found inhabiting any of the tubes. The common eastern dogwhelk *Nassarius vibex* was present in these clusters in large numbers (up to 15, mean = 7). Two species of mussels occurred on these assemblages, the tulip mussel *Modiolus americanus* (Leach, 1815) and the lateral mussel *Musculus lateralis* (Say, 1822). A small predatory mudcrab, *Neopanope sayi* (Smith, 1869), barnacles, and the polychaete worm *Nereis succinea* were also found. All associated fauna represent common species in coastal Georgian waters.

Males dominated the sample with a sex ratio of 1.53:1 (M:F). The range of shell lengths of the female arks examined was 6.79 to 19.21 mm, and was 7.15 to 21.44 mm for males. A size frequency distribution for males and females combined is presented in Figure 2. Gonadal development was apparent in every specimen examined, indicating a very small size at first maturity. A partially spawned stage dominated in both sexes (females 49%; males 53%). In females the spent stage was the next most common with 37% of individuals having spawned most of their gonadal material. For males the ripe stage was the second most dominant stage with 44% of the 75 individuals. The remainder was split between 4% in the early active, 2% in the late active, and 8% in the ripe stage for females, and 3% in the spent stage for males. Figures 1B and C show histological sections of the gonads of ripe male and female cut-ribbed arks, respectively.

Sexual dimorphism was not evident from the regression equations based on the morphological data: female width = 0.48 (female length) + 0.16, r = 0.94; male width = 0.44 (male length) + 0.65, r = 0.96: female height = 0.59 (female length) + 0.47, r = 0.96; male height = 0.59 (male length) + 0.57, r = 0.95: female weight = 0.11 (female length) – 1.02,

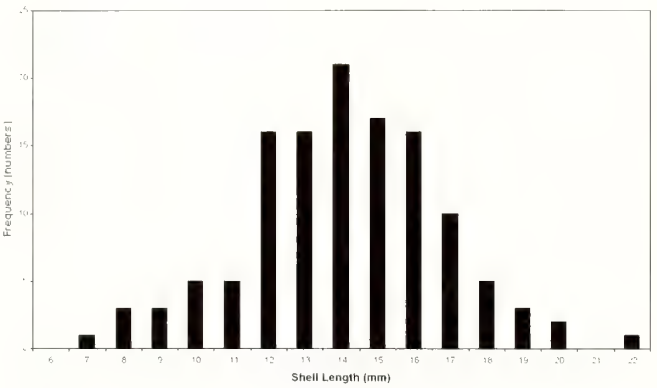

Figure 2. Size frequency distribution (shell length, in mm) of cut-ribbed arks (*Anadara floridana*) used in the histological analysis of the size at sexual maturity.

r = 0.94; male weight = 0.11 (male length) – 1.01, r = 0.94. Shell length varied from 6.79 to 21.44 mm (mean ± S.E. = 13.5 ± 0.2), width from 3.18 to 9.98 mm (6.60 ± 0.11), height from 4.60 to 12.75 mm (8.52 ± 0.14), and weight from almost 0 to 1.70 g (0.50 ± 0.03).

DISCUSSION

In marine environments dominated by soft sediment, molluscan and crustacean shells can provide the only favorable hard surface for settling organisms to colonize (Federighi 1931, MacKenzie 1962, Pearse and Thorson 1967, Cake 1983, Olabarria 2000). Therefore, it should come as no surprise to find these young arks on whelk shells in the muddy and sandy substrates of coastal Georgia. In fact, this is not the first time that clusters of arks have been reported on whelks here; knobbed whelks were previously observed carrying blood arks (*Anadara ovalis*) in the surf zone on St. Simons Island (Walker 2000). Whelks are a prime basibiont, being locally abundant and typically spending large portions of time quiescent and partially buried in the substrate.

What is interesting about our observations in the laboratory is that young cut-ribbed arks can be highly mobile. It is possible that these arks actively crawled onto their hosts' shells from the neighboring soft seafloor where they originally settled, rather than settling directly out of the water column onto the hosts' shells. Similar crawling behavior has been noted by the authors for young transverse arks, *Anadara transversa* (Say, 1822), which were also noted to actively climb the sides of holding tanks (Walker and Power 2004). There is limited information on the settling behavior of ark species. Hatchery experiments with the Sydney cockle, *Anadara trapezia* (Deshayes, 1840), failed to induce metamorphosis onto upweller screens within a two-week period (Nell *et al.* 1994). Species of Arcidae of commercial importance in Malaysia are reported to settle onto fine muddy bottoms with gradual slopes around the mouth of rivers (Tookwinas 1985).

Although we do not have any information on the specific growth rates of *Anadara floridana*, we do know that *Anadara ovalis* can reach 10 to 12 mm in length in about 8 months in local waters (Power and Walker 2002). If *A. floridana* exhibits similar growth rates, most of the arks found on whelks in the present study (mean of approximately 18 mm) could be as much as one year old. It is unlikely that they could have persisted on the shells for such an extended period because whelks aggregate to spawn during the spring and fall months, bury completely during the coldest winter months, and often enter the intertidal zone in search of food on oyster reefs (Walker 1988).

Transport via the mobile whelk has the potential to increase feeding opportunities both through currents generated by the physical movement of the host and also by the potential to disperse to more favorable locations. The clusters can also provide refuges for the smaller arks, which were observed within the masses of byssal threads. Possible effects upon the whelk host include a slower crawling speed and reduced burying capabilities, which together could result in an increased vulnerability to predation (Overstreet 1983).

Although there would be no transportation advantage to clustering around tubeworms, the tubes are raised off the substrate and somewhat rigid, so attachment may enhance filter-feeding. In all the clusters observed, the largest arks were those on the outside; these presumably had greater feeding opportunities and growth rates. Cut-ribbed arks are also reported to settle on blades of turtle grass (*Thalassia testudinum*) (Bologna and Heck 2000) and on live and dead shells of the calico scallop, *Argopecten gibbus* (Linnaeus, 1758), as well as on other shell debris from commercial scallop beds (R. L. Walker, pers. obs.). These structures provide stable attachment sites for the young arks. No living polychaetes were found inhabiting any of the tubes; it is likely that they died from a lack of oxygen and an inability to feed once the arks clustered and grew around them. Unlike the whelk hosts, the tubeworms would have shared the same trophic requirements as the arks and would have been food-limited at the center of the cluster (Olabarria 2000).

Many members of the Arcidae have been reported to initiate gametogenesis and reach sexual maturity at a small size (Power and Walker 2002). Sexual maturity in the transverse ark is attained at 10 mm and 12 mm for male and female arks, respectively (Walker and Power 2004). Similarly, the blood ark matures at a relatively small size, at 9.9 mm and 11.5 mm for male and female arks, respectively (Power and Walker 2002). The smallest male and female cut-ribbed arks examined in the present study measured 7.1 and 6.8 mm, respectively, and both were sexually mature, indicating that the largest North American ark species matures at one of the smallest sizes. Through mortality these arks are likely to occur in reduced densities once they get older, therefore the early gametogenic development and clustering behavior observed may be an effective reproductive strategy.

Partially spawned individuals dominated the reproductive stages identified; however, this does represent a single temporal sample. Spawning patterns for many ark species are less confined to a single narrow season with decreasing latitude, and some even employ a "dribble" spawning approach wherein different portions of the gonads develop at different times and release their gametes over an extended period (Power *et al.* 2004).

In this study, males dominated the population of arks, with an overall sex ratio of 1.53:1.00 (M:F). Higher male to

female ratios have been documented for blood arks from Georgia (Power and Walker 2002), Florida (Power *et al.* 2004), and Virginia (McGraw *et al.* 1998). An equal sex ratio has been observed in *Anadara granosa* (Linnaeus, 1758) (Pathansali 1966, Broom 1983) and *Anadara subcrenata* (Lischke, 1869) (Ting *et al.* 1972), but females have been more abundant in *Anadara transversa* (Walker and Power 2004) and *Anadara senilis* (Linnaeus, 1758) (Yoloye 1974). Hermaphroditic arks are rare and none were observed in the present study. However, we only examined young arks in the size range 6.79 to 21.44 mm in length. Baron (1992) found males dominating the smaller size-classes of *Anadara scapha* (Linnaeus, 1758) while females were more frequent in the larger size-classes. Because individuals of *Anadara floridana* have been recorded up to 127 mm in length (Abbott 1974), the possibility for protandric hermaphroditism in this species cannot be ruled out.

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LITERATURE CITED

Abbott, R. T. 1974. *American Seashells*, Second Edition. Van Nostrand Reinhold Co., New York.

Baron, J. 1992. Reproductive cycles of the bivalve mollusks *Atactodea striata* (Gmelin), *Gafrarium tumidum* (Roding) and *Anadara scapha* (L.) in New Caledonia. *Australian Journal of Marine and Freshwater Research* **43**: 393-402.

Baqueiro, E. C. 1989. Clam culture in Mexico: Past, present and future. *In*: J. J. Manzi and M. A. Castagna, eds., *Clam Mariculture in North America*. Elsevier, New York. Pp. 383-394.

Baqueiro, E. C., M. D. Mucino, and R. M. Merino. 1982. Situación de una población de pata de mula *Anarara tuberculosa* sujeta a explotación intensiva en la Bahía de la Paz, Baja California Sur, México. *Ciencia Pesquera, Instituto Nacional de la Pesca, México* **3**: 75-82.

Bologna, P. A. and K. L. Heck. 2000. Impacts of seagrass habitat architecture on bivalve settlement. *Estuaries* **23**: 449-457.

Broom, M. J. 1983. Gonadal development and spawning in *Anadara granosa* (L.) (Bivalvia: Arcidae). *Aquaculture* **30**: 211-219.

Broom, M. J. 1985. *The Biology and Culture of Marine Bivalve Mollusc of the Genus* Anadara. ICLARM, International Center for Living Aquatic Resources Management, Manila, Philippines. *ICLARM Studies and Reviews* 12, Contribution No. 263.

Cake, E. W. 1983. Symbiotic associations involving the Southern oyster drill *Thais haemastoma floridana* (Conrad) and macrocrustaceans in Mississippi waters. *Journal of Shellfish Research* **3**: 117-128.

Federighi, H. 1931. Studies of the oyster drill (*Urolsalpinx cinerea* Say). *U.S. Bureau of Commercial Fisheries Bulletin* **47**: 85-115.

Howard, D. W. and C. S. Smith. 1983. *Histological Techniques for Marine Bivalve Mollusks*. NOAA Technical Memorandum NMFS-F/NEC-25. Washington, D.C.

MacKenzie, C.L. 1962. Transportation of oyster drills by horseshoe crabs. *Science* **137**: 36-37.

Manzi, J. J. and M. A. Castagna. 1989. *Clam Mariculture in North America*. Elsevier, New York.

McGraw, K. A. and M. A. Castagna. 1994. *Some Observations on Arkshell Clams*, Noetia ponderosa *and* Anadara ovalis, *and Implications for Fisheries Management.* Virginia Sea Grant College Program, Technical Report VSG-94-11.

McGraw, K. A., M. A. Castagna, and S. D. Dennis. 1996. *Population Structure of the Arkshell Clams* Noetia ponderosa *and* Anadara ovalis *in the Oceanside Lagoons and Tidal Creeks of Virginia and Implications for Fisheries Management.* Final Report submitted to Saltonstall-Kennedy Program Office of the National Oceanic and Atmospheric Administration, National Marine Fisheries Service, NOAA Grant No. NA46FD0339.

McGraw, K. A., M. A. Castagna, and S. D. Dennis. 1998. *The Arkshell Clams* Noetia ponderosa *and* Anadara ovalis *in the Oceanside Lagoon System of Virginia: A Study of Predation, Reproductive Biology and Condition Index.* Final Report submitted to Saltonstall-Kennedy Program Office of the National Oceanic and Atmospheric Administration, National Marine Fisheries Service, NOAA Grant No. NA66FD0010.

Nell, J. A., W. A. O'Connor, M. P. Heasman, and L. J. Goard. 1994. Hatchery production for the vererid clam *Katelysia rhytiphora* (Lamy) and the Sydney cockle *Anadara trapezia* (Deshayes). *Aquaculture* **119**: 149-156.

Nie, Z.-Q. 1990. The culture of marine bivalve mollusks in China. *In*: R. W. Menzel, ed., *Estuarine and Marine Bivalve Culture,* CRC Press, Inc., Boston. Pp. 261-276.

Olabarria, C. 2000. Epibiont mollusks on neogastropod shells from sandy bottoms, Pacific coast of Mexico. *Journal of the Marine Biological Association of the United Kingdom* **80**: 291-298.

Overstreet, R. M. 1983. Metazoan symbionts of crustaceans. *In*: D. E. Bliss, ed., *The Biology of Crustacea*, Volume 6, New York Academic Press. Pp. 155-250.

Pathansali, D. 1966. Notes on the biology of the cockle. Proceedings of the Indo-Pacific Fisheries Council **11**: 84-98.

Pearse, J. B. and G. Thorson. 1967. The feeding and reproductive biology of the red whelk, *Neptunea antiqua* (L.) (Gastropoda: Prosobranchia). *Ophelia* **4**: 227-314.

Power, A. J. and R. L. Walker. 2002. Growth and gametogenic cycle of the blood ark, *Anadara ovalis* (Bruguière, 1789) in coastal Georgia. *Journal of Shellfish Research* **21**: 157-162.

Power, A. J., J. Nunez, M. Mitchell, and R. L. Walker. 2004. Reproductive pattern of the blood ark, *Anadara ovalis* from the northeast coast of Florida. *Journal of Shellfish Research* **23**: 173-178.

Spruck, C., R. L. Walker, M. Sweeney, and D. Hurley. 1994. Gametogenic cycle in the non-native Atlantic surfclam, *Spisula solidissima* (Dillwyn, 1817), cultured in the coastal waters of Georgia. *Gulf Research Reports* **9**: 131-137.

Ting, Y., S. Kasahara, and N. Nakamura. 1972. An ecology study of the so-called Mogai *Anadara subcrenata* (Lischke) cultured in Kasaoka Bay. *Journal of Fisheries and Animal Husbandry* **11**: 91-110.

Tookwinas, S. 1985. Commercial cockle farming in southern Thailand. *International Center for Living Aquatic Resources Management Translations* **7**: 1-13 [E. W. McCoy, translator].

Umezawa, S. 1992. Experimentation to improve recruitment of blood ark shell, *Scapharca broughtonii*, in the Seto Inland Sea. NOAA Technical Report, National Marine Fisheries Service **106**: 105-114.

Walker, R. L. 1988. Observations on intertidal whelk (*Busycon* and *Busycotypus*) populations in Wassaw Sound, Georgia. *Journal of Shellfish Research* **7**: 473-478.

Walker, R. L. 2000. Living precariously in the food chain. Marinet, *Newsletter of the Marine Extension Service Savannah, Georgia* **11**: 1-4.

Walker, R. L. and P. B. Heffernan. 1994. Temporal and spatial effects of intertidal exposure on the gametogenic cycle of the northern, *Mercenaria mercenaria* (Linnaeus, 1758), in coastal Georgia. *Journal of Shellfish Research* **13**: 479-486.

Walker, R. L. and A. J. Power. 2004. Growth and gametogenic cycle of the transverse ark, *Anadara transversa* (Say, 1822), in coastal Georgia. *American Malacological Bulletin* **18**: 55-60.

Yoloye, V. 1974. The sexual phases of the West African bloody cockle *Anadara senilis* (L.). *Proceedings of the Malacological Society of London* **41**: 25-27.

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Seasonal growth and mortality of juveniles of *Lampsilis fasciola* (Bivalvia: Unionidae) released to a fish hatchery raceway

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Abstract: Recent efforts to restore remnant or extirpated populations of freshwater mussels have focused on artificial propagation as an effective and practical conservation strategy. Although artificially cultured juveniles have been produced and released to the wild at various times of the year, no study has investigated the best time of year to release these juveniles. Newly metamorphosed juveniles of the wavyrayed lampmussel (*Lampsilis fasciola*) were released into a stream-fed fish hatchery raceway during March, June, and September. Growth and survival rates were measured 32, 52, 72, and 92 days post-metamorphosis. Juveniles released in June experienced the greatest growth and survival rates. Juveniles released in September and March experienced high mortality within the first month of release and exhibited poor growth in the cool water conditions typical of those seasons. Overwinter survival exhibited a size-dependent relationship.

Key words: *Lampsilis fasciola*, life stage, propagation, overwinter mortality, temperature

Over 70% of North American's freshwater mussel species are considered extinct, endangered, threatened, or of special concern (Williams *et al.* 1993). Overharvesting, pollution, habitat destruction, and the introduction of exotic species have destroyed or altered much of the habitat that once supported a high diversity and abundance of freshwater mussels. As a result of increased environmental protection over the last thirty years, some stream reaches are beginning to recover from the perturbations that occurred over the past century. Some of these recovering stream reaches are currently or anticipated to become suitable habitat for mussel re-colonization. However, the fecundity and density of many mussel populations has been reduced to the point that natural re-colonization is unlikely. Many researchers and resource managers have focused on the use of laboratory-cultured mussels as one method to augment or reestablish these populations. However, the viability of such a technique for successful re-colonization remains severely understudied. One variable that may strongly influence the success or failure of restoration efforts may be the time of year juvenile mussels are released to the natal streams.

In this study, we tested the release of newly metamorphosed juvenile mussels into a fish raceway, simulating natural stream conditions, at three times of the year to identify the optimal time for mussel recruitment.

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METHODS

We conducted tests in a fish raceway at the Buller Fish Culture Station near Marion, Virginia, U.S.A. Hatchery water originated from the South Fork Holston River (SFHR) at river kilometer 169 and was piped from an instream impoundment into a 2 ha pond prior to diversion to the raceway. The water from the pond was warmer and richer in algae than that in the SFHR, a coolwater trout stream. The raceway was 18 m long and was partitioned longitudinally with plywood (60 cm wide by 18 mm thick), forming 4 separate 0.6 m wide sub-raceways. Each sub-raceway was divided horizontally into 1.2 m long units using 3 mm mesh galvanized screen to provide designated areas for various experiments. A maximum flow of 720 L/min (velocity = 0.13 cm/sec) and a water depth of 29 cm was maintained throughout the study.

We selected the wavyrayed lampmussel (*Lampsilis fasciola* Rafinesque, 1820) for use in this study because populations of this species occur naturally in the Holston River drainage and gravid females of this species can be collected throughout most of the year. In addition, previous studies have confirmed suitable fish hosts for *L. fasciola* (Zale and Neves 1982) and have successfully reared significant numbers of juveniles under laboratory conditions (O'Beirn *et al.* 1998).

We collected gravid mussels by snorkeling at three locations in the Clinch River basin (Indian Creek, Tazewell County, Virginia, Clinch River at Nash Ford, Russell County, Virginia, and Clinch River at Hancock County,

Tennessee). Mussels were transported in river water to the laboratory in an aerated cooler and held in a Living Stream (Frigid Units, Inc., 3214 Sylvania Ave., Toledo, Ohio) maintained at 15°C.

We infested hatchery-reared largemouth bass (*Micropterus salmoides* Lacepède, 1802) (13 cm in length) with glochidia extracted from collected mussels using the procedure described by Zale and Neves (1982). Once infested with glochidia, we returned fish to a tank (100 L) supplied by recirculating water. After 2 weeks, the holding tank was checked daily for metamorphosed juvenile mussels by siphoning the bottom of the tank and capturing juveniles in a 130 μm nylon mesh sieve.

We released propagated juveniles to the hatchery raceway on 26 June and 16 September 1998, and 8 March and 16 September 1999. With the exception of the September 1998 trial, we extracted glochidia from a minimum of 4 gravid females to produce a pooled stock of juveniles for each trial. Because only 1 gravid female was available for the September 1998 release, we repeated this trial in September 1999. For each trial, 500 newly metamorphosed juveniles (<2 days old) were used, and a sub-sample of 10 randomly selected individuals was measured for initial mean shell length using a calibrated ocular micrometer and a stereozoom dissecting microscope. After capture, we immediately transported juvenile mussels in a 1 L container of well water to the hatchery. At the hatchery, we placed 50 juveniles in each of ten 200 mL plastic containers (8 cm^2 and 3.5 cm deep), each containing 5 mm of limestone sand (1 > 2.5 mm). We partially submerged dishes in hatchery water to allow juveniles to acclimate to the water temperatures. After a 2 h acclimation period, all dishes were submerged within a designated area of the raceway. Each dish was covered with a 120 μm mesh nylon screen over each dish to prevent the loss of juveniles during submersion. Once each dish was placed at the bottom of the raceway and the contents of each dish had settled, the screen was removed. An Onset Optic Stowaway data logger (Onset Computer Corporation, 536 MacArthur Blvd., P.O. Box 3450, Pocasset, Massachusetts) was placed in the raceway to record temperature hourly.

We sampled dishes for juvenile mussels at 32, 52, 72, and 92 days to obtain trends in growth and survival over time. Juveniles introduced to the raceway in June were sampled periodically after 92 days to collect subsequent information on growth and survival. Because sampling may negatively affect juvenile growth (O'Beirn *et al.* 1998), we reduced our sampling efforts by systematically selecting 5 out of the 10 dishes during each sampling period to evaluate growth and survival. Subsets (5 replicates) were alternately sampled for subsequent sampling periods. During sampling events, each selected dish was removed from the raceway and the contents were decanted through two different mesh sieves (1000 μm, then 120 μm). Samples were rinsed with hatchery water for 1 min to separate juveniles from the substratum. Sediment that naturally accumulated in each dish was discarded during the rinsing process, and the limestone sand (retained in the 1000 μm sieve) was returned to the dish. Juveniles were rinsed from the lower sieve (120 μm) into a petri dish.

We counted live juveniles, with the aid of a stereozoom microscope, to determine survival in each dish. Mean shell length was calculated in each dish with measurements obtained from 10 randomly selected juveniles. Once growth and survival data were obtained for each dish, we placed juvenile mussels into their original dish and returned each dish to the raceway. Because juveniles occasionally escaped from dishes, we vacuumed the bottom of the raceway unit during each sampling period with a shop vacuum to collect emigrant juveniles. The vacuumed matter was poured through a graduated series of sieves to collect juveniles. We assumed that the rate at which juveniles escaped was equal among replicates. Therefore, we reallocated migrant juveniles equally among the 10 dishes and survival was adjusted accordingly. Although we recognized this assumption to be crude, we believe the inclusion of migrant juveniles produced a more accurate analysis of mean survival.

Due to limitations in comparable data and statistical complications caused by escaped juveniles, only data for the 32-day growth and survival were used for statistical comparison among released cohorts. We used a one-way ANOVA followed by Fisher's pairwise comparisons to compare 32-day growth and survival among juvenile mussels released at different times of the year. All statistical tests were performed with the SAS statistical package (SAS Institute, Inc., Cary, North Carolina). Subsequent data were used to illustrate trends in growth and survival over time. We also compared sample variances and used a Chi-square test to compare the growth of June-release mussels measured at 122 days (Fall) and 334 days (Spring) to evaluate size-selective mortality over the winter months (when water temperatures remain below 15°C).

RESULTS

Survival was significantly different at 32 days among juveniles released at different months ($p < 0.0001$) (Fig. 1). Juveniles released in June exhibited the highest survival rate and 13% of these juveniles survived through the subsequent winter months. Presumably as a result of error in systematic sampling, mean survival in June appeared higher at 92 days (48%) than at 72 days. However, June-release survival data among the 52, 72, and 92-day sampling periods were not statistically significant (ANOVA; $p = 0.122$). Juveniles released in September and March exhibited significant mor-

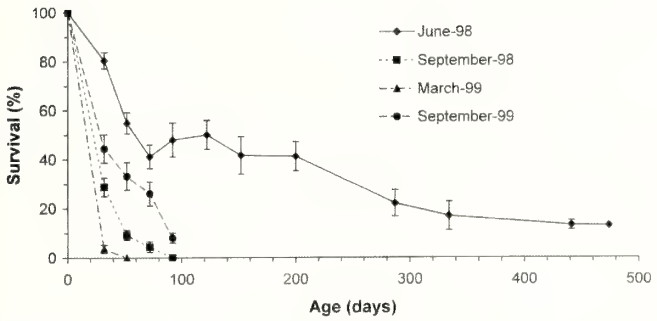

Figure 1. Survival (mean ± SE) of juveniles of the wavyrayed lampmussel, *Lampsilis fasciola*, released in a fish hatchery raceway during June and September 1998 and March and September 1999.

tality within the first 32 days post-metamorphosis, with only a few survivors reported at 92 days from the September 1999 release.

Juvenile mussels released at different months showed significant differences in mean shell length at 32 days ($p < 0.0001$) (Fig. 2). Fisher's pairwise comparisons revealed that shell length at 32 days was greater for mussels released in June and least for those released in March. Trends in growth of juveniles released in September 1998 and September 1999 were almost identical. Growth of juveniles released in June was minimal between mid-October to May when water temperatures remained below 15°C, but then increased rapidly from May onward until termination of the study in October 1999. Based on growth values of individual juvenile mussels obtained at 122 and 334 days, pre-winter and post-winter mean shell lengths (mean ± SE) were 1.73 ± 0.06 mm (n = 50, range 1.08-2.92 mm, variance 0.21) and 1.92 ± 0.06 mm (n = 31, range 1.33-2.56 mm, variance 0.10), respectively. Based on 10 categories of shell length, the frequency distribution of the sizes of sampled juveniles was significantly different between 122 days and 334 days ($df = 9$, $\chi^2 = 20.41$, $p \leq 0.025$).

Of the juveniles released in June, 9.6%, 15.1%, 8.1% and 1.0% were collected outside of replicate containers at 72, 92, 122, and 152 days, respectively. Little escapement was evident in other trials, with < 1% observed during any given sampling period. During the 92-day sampling period, mean shell length of June-released juveniles collected outside of containers (1.81 ± 0.28 mm) was significantly greater ($p <$

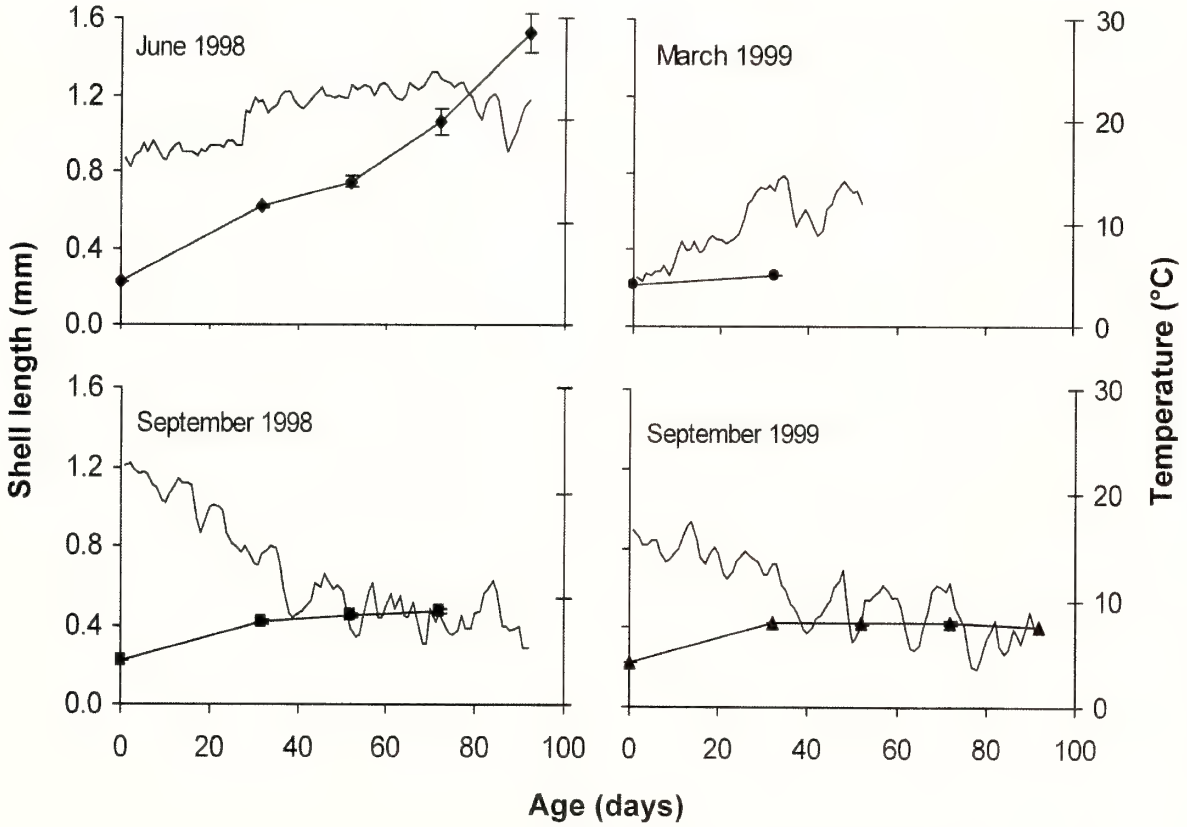

Figure 2. Daily mean water temperatures (°C) and mean shell lengths (mm ± SE) of newly metamorphosed juveniles of the wavyrayed lampmussel, *Lampsilis fasciola*, released in the fish hatchery raceway during June and September 1998 and March and September 1999.

0.0001) than the mean shell length of juveniles that remained in the containers (1.45 ± 0.30 mm).

DISCUSSION

Like most long-term brooders, individuals of *Lampsilis fasciola* spawn in the late summer and brood glochidia over the winter. Gravid females are typically found actively displaying their mantle lure in spring and early summer, and are not commonly found gravid from mid-summer to early fall. Although a small percent of glochidia may be released in mid-summer, most glochidia of *L. fasciola* are released from mid-spring to early summer (Zale and Neves 1982). Glochidia that are released and attach to host fish early in the year (winter and early spring) will probably remain encysted within the gill tissue of the host fish until water temperatures increase in mid-spring (Watters and O'Dee 1999). This brooding cycle ensures that the majority of newly metamorphosed juveniles will excyst from host fish at an opportune time to maximize growth prior to winter.

Results of our study concur with this natural spawning and brooding cycle, and suggest that the success of age-0 recruitment is, in part, dependent on the time of year that juvenile mussels are released. Growth and survival declined precipitously when juveniles were released at cooler water temperatures. An estimated 13% of juveniles released during June were able to survive their first winter, whereas juveniles released in September did not survive the winter, and those released in March did not survive beyond 52 days. Beaty and Neves (2004) reported similar findings with individuals of *Villosa iris* (I. Lea, 1829) cultured in natural river water; juveniles exhibited greater growth and survival when released to the culture system in June compared to subsequent trials initiated later in the summer.

These differences in survival may be influenced by seasonal opportunities for growth and size-selective overwinter mortality. In situations where the possibility of actual growth can be ruled out, an increase in mean size and decrease in variance over winter typically indicate mortality of smaller individuals (Munch *et al.* 2003). Mean shell length of juveniles released in June attained 1.73 mm in mid-October (122 days) and increased over the winter months, reaching a mean of 1.92 mm in late April (334 days). Given the significant decrease in variance in shell length over winter, and assuming that juveniles of *Lampsilis fasciola* do not grow appreciably during winter (water temperature remains below 15°C) (Beaty and Neves 2004), this apparent overwinter increase in mean shell length indicates size-selective overwinter mortality.

Buddenseik (1995) reported that the overwinter survival of age-0 juveniles of *Margaritifera margaritifera* (Linnaeus, 1758) was size-dependent. One hundred percent of juveniles of *M. margaritifera* less than 700 μm in shell length died during winter months, and only juveniles greater than 900 μm had a 50% chance of surviving through the winter. Similarly, our juveniles released in September and March exhibited minimal growth and were unable to survive more than 2 months. Similar size-dependent relationships have been reported in studies of marine bivalves (Beal *et al.* 1995) and many fish species (Toneys and Coble 1979, Gutreuter and Anderson 1985, Post and Evans 1989, McGovern and Olney 1996, Hurst and Conover 1998, Munch *et al.* 2003). In fish, larger members of a cohort possess greater energy reserves and usually exhibit a significant survival advantage over smaller members of the same cohort (Paloheimo and Dickie 1966). Furthermore, weight-specific metabolic costs are usually reduced in larger individuals (Werner and Gilliam 1984). Mortality during the first winter is thought to be a pivotal determination of cohort abundance in many taxa (Munch *et al.* 2003) and is likely to be for young unionids as well.

Based on results of these rearing trials, juvenile mussels of the species *Lampsilis fasciola* will likely have the best opportunity for growth and survival if released to natal streams in spring to early summer when average daily water temperatures exceed 15°C. This window of opportunity may be the same for species with a similar brooding cycle as *L. fasciola*.

Although juveniles that escaped containers were not incorporated into our statistical analysis, we believe the following observation is worth noting. Juveniles collected outside of containers were on average 25% larger than juveniles that remained in the containers at 92 days post-metamorphosis. However, it is not known whether juveniles that escaped from containers were larger, and therefore had a greater capability to escape, or whether their greater size resulted from better food and growth conditions outside of the containers. Greater mobility in the raceway may enhance a juvenile's ability to pedal feed and seek food concentrations not available within the containers. Higher juvenile densities within the containers may also be a contributing factor in retarding growth rates. However, Beaty and Neves (2004) found no significant differences in growth and survival among different stocking densities of juvenile mussels of *Villosa iris* held in their flow-through culture system. Additional investigation of these potential influences will be necessary to further our understanding of the niche requirements for early life stages.

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LITERATURE CITED

Beal, B. F., C. D. Lithgow, D. P. Shaw, S. Renshaw, and D. Ouellette. 1995. Overwintering hatchery-reared individuals of the soft-shell clam, *Mya arenaria* L.: A field test of site, clam size, and intraspecific density. *Aquaculture* **130**: 145-158.

Beaty, B. B., and R. J. Neves. 2004. Use of a natural river water flow-through culture system for rearing juvenile freshwater mussels (Bivalvia: Unionidae) and evaluation of the effects of substrate size, temperature, and stocking density. *American Malacological Bulletin* **19**: 15-23.

Buddensiek, V. 1995. The culture of juvenile freshwater pearl mussels *Margaritifera margaritifera* L. in cages: A contribution to conservation programmes and the knowledge of habitat requirements. *Biological Conservation* **74**: 33-40.

Gutreuter, S. J. and R. O. Anderson. 1985. Importance of body size to the recruitment process in largemouth bass populations. *Transactions of the American Fisheries Society* **114**: 317-327.

Hurst, T. P. and D. O. Conover. 1998. Winter mortality of young-of-the-year Hudson River striped bass (*Morone saxatilis*): Size dependent patterns and effects on recruitment. *Canadian Journal of Fisheries and Aquatic Sciences* **55**: 1122-1130.

McGovern, J. C. and J. E. Olney. 1996. Factors affecting survival of early life stages and subsequent recruitment of striped bass on Pamunkey River, Virginia. *Canadian Journal of Fisheries and Aquatic Sciences* **53**: 1713-1726.

Munch, S. B., M. Mangel, and D. O. Conover. 2003. Quantifying natural selection on body size from field data: winter mortality in *Menidia menidia*. *Ecology* **84**: 2168-2177.

O'Beirn, F. X., R. J. Neves, and M. B. Steg. 1998. Survival and growth of juvenile freshwater mussels (Unionidae) in a recirculating aquaculture system. *American Malacological Bulletin* **14**: 165-171.

Paloheimo, J. R. and L. M. Dickie. 1966. Food and growth in fishes. II. Effects of food and temperature on the relation between metabolism and body weight. *Journal of the Fisheries Research Board of Canada* **23**: 869-908.

Post, J. R., and D. O. Evans. 1989. Size-dependent overwintering mortality of young-of-the-year yellow perch (*Perca flavescens*): laboratory, in situ enclosure, and field experiments. *Canadian Journal of Fisheries and Aquatic Sciences* **46**: 1958-1968.

Toneys, M. L. and D. W. Coble. 1979. Size-related, first winter mortality of freshwater fishes. *Transactions of the American Fisheries Society* **108**: 415-419.

Watters, G. T. and S. H. O'Dee. 1999. Glochidia of the freshwater mussel *Lampsilis* overwintering on fish hosts. *Journal of Molluscan Studies* **65**: 453-459.

Werner, E. E. and J. F. Gilliam. 1984. The ontogenetic niche and species interactions in size-structured populations. *Annual Review of Ecology and Systematics* **15**: 393-425.

Williams J. D., M. L. Warren, K. S. Cummings, J. L Harris, and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. *Fisheries* **18**: 6-22.

Zale, A. V. and R. J. Neves. 1982. Fish hosts of four species of lampsiline mussels (Mollusca: Unionidae) in Big Moccasin Creek, Virginia. *Canadian Journal of Zoology* **60**: 2535-2542.

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Strategies for sustainable dye harvest of the purple conch *Plicopurpura pansa* (Gould, 1853) from west central Mexico

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Abstract: The purple conch, *Plicopurpura pansa*, occurs on rocky shores of the tropical eastern Pacific of North and Central America, ranging from Magdalena Bay, Baja California to Colombia. When disturbed, it exudes a secretion that photo-oxidizes to an intense purple hue. This product has been used as a dye for ceremonial dresses. Unlike the case in other dye-producing molluscs, it is not necessary to sacrifice individuals to obtain the dye, allowing repetitive "milking" of the same animal without causing mortality. Evaluation of population parameters, rates of dye produced as a function of age at first milking, and milking frequency allowed us to simulate different exploitation scenarios and to evaluate strategies to determine the most profitable exploitation intensity and optimum milking frequencies. Laboratory experiments and field data showed that mortality occurs when the interval between milking is lower than 21 days. The most profitable exploitation strategies suggest that up to 310 L of dye in 50 km of shoreline length can be harvested during the three-month milking season. This volume of dye is sufficient to stain up to 260 skeins of cotton thread. Simulations showed that the stocks can withstand sustainable exploitation along their distribution range, allowing commercial exploitation of the dye produced by stocks of purple conch in other areas apart from those where it currently takes place, benefiting other groups of fishers elsewhere and ensuring the conservation of this tradition.

Key words: Population dynamics, conservation, resource management, bioeconomic model, simulation, sustainable yield.

The purple conch *Plicopurpura pansa* (Gould, 1853) is well known for the purple dye it produces. It is an abundant gastropod mollusc along the intertidal rocky shores of the Panamic Province of the eastern Pacific, ranging from "Magdalena Bay Baja California, through the south end of the Gulf and south to Colombia and the Galapagos Islands, common on rocks in exposed locations"(Keen 1971: 553). In contrast with other molluscs that produce dye (species of *Murex, Purpura,* and *Thais*), in which it is necessary to break the shell to extract the dye-producing gland, in *P. pansa* milking can be done by stimulating the foot; killing the animal is not necessary. This allows successive milking of the same animal as long as it is carefully handled and allowed at least 21 days for recovery before milking again to avoid mortality induced by handling (Michel-Morfín and Chávez 2000).

Most skeins are made out of cotton, but sometimes the material is silk; each one weights 285 g (Michel-Morfín *et al.* 2002b). Staining of cotton skeins is carried on at the coast right after removing conchs from the rocks, where the dye is drained directly on the skein. Once this is done, the conch is deposited on its place on the intertidal rocks. Ceremonial dresses, used mainly by women, are stained with the purple dye, as well as with other natural colors, such as the red dye extracted from an insect parasite of *Opuntia* sp. and the indigo dye from some plants.

This paper synthesizes the information formerly published by the authors working with the purple conch and dye yield (Michel-Morfín and Chávez 2000), population dynamics (Michel-Morfín *et al.* 2000), and fecundity and egg morphometry of wild stocks (Michel-Morfín *et al.* 2002a). We examined the population dynamics of this mollusc to evaluate harvesting strategies within the framework of sustainable exploitation for the preservation of both cultural tradition as well as this natural resource.

MATERIALS AND METHODS

Population parameters including growth rate and natural mortality, volume of dye yield as a function of shell length and milking frequency, "fishing" mortality (induced by handling and milking), as well as some economic data, including the costs of milking and staining skeins of cotton thread and values of dye and stained cotton skeins, were evaluated previously. The results are described in the papers by Ríos-Jara *et al.* 1994, Michel-Morfín and Chávez 2000, Michel-Morfín *et al.* 2000, and Michel-Morfín *et al.* 2002b. Values of parameters and sex ratios used in the model are indicated in Table 1.

Shell length and volume of dye produced

Parameter values allowed simulation of harvest sce-

Table 1. Values used for the simulation model. Data were obtained after sampling 13 sites in 5 states in western Mexico. Transect size was a costal band 400 m long by 2 m wide. Extension of the coastline used for reference is 50 km long by 2 m wide. Parameter values of the von Bertalanffy growth model $\{1 = L_\infty[1 - \exp^{(-K(t-t_0))}]\}$; *a* and *b* of the allometric equation describing the length-weight relationship ($W_\infty = aL^b$); natural mortality coefficient (M), and α and β of the power regression describing the relation between conch shell length (L) and volume of dye produced (V), $V = \alpha L^\beta$ are given below. t, time units (years). Source data: Michel-Morfín *et al.* (2000, 2002b). Some values related to costs and benefits in USD are added.

Attributes (units)	Females	Males	General
L_∞ (mm)	110	102	
K (yr^{-1})	0.27	0.21	
t_0 (yr)	−0.04	−0.04	
W_∞ (g)	198	134	
a	0.0003	0.0002	
b	2.85	2.90	
M (yr^{-1})*	0.405	0.315	
α	0.001	0.0005	
β	2.43	2.06	
R_{max}**	63.25	47.31	
b**	0.7914	0.5964	
*a***	0.25	0.25	
Mean density			1.1
Standard error of mean density:			0.114
Female:Male ratio			1.0:1.13
Daily cost of milking and staining skeins per person			100
Costs per one liter of dye produced (USD)			25
Value of one liter of dye (USD)			75

* Estimated as M = 1.5K, a Beverton invariant (Jensen 1996, 1997)

** Recruitment model parameters

narios and optimization of harvesting strategies, including yield of dye, optimized milking schedule, and the highest frequency of milking, as well as minimum shell lengths to be milked without causing mortality due to handling and physiological exhaustion. Previous experiments (Michel-Morfín and Chávez 2000) provided data that allowed us to derive an equation describing the volume of dye produced in ml (V_{MF}) and the proportion of survival with respect to milking frequency in days (D). The accumulated mean production of dye per conch over the three-month milking season decreased linearly and survival increased from 75% at a milking frequency of seven days to 100% at 28 days. When the two variables (accumulated dye milked over three months and milking frequency) were combined, the equation describing this process was the following:

$$V_{MF} = 0.0066D^3 - 0.41D^2 + 6.47D + 27.3$$

Simulation models have been developed and applied to describe and evaluate optimum yields and optimum harvesting strategies in many fisheries. Some of them (Grant 1986, Die and Watson 1992, Prince 1992, Chávez 1994, 1996, Chávez and Arreguín-Sánchez 1994, Cruz-Romero *et al.* 1996, Chávez 1996) served as a basis for the the model we developed for purple conch.

In contrast to most fisheries, for purple conch the harvest of animal tissue is not the goal of exploitation. For this reason, substitution of dye yield for typical biomass yield was made in the catch equation and the model was validated for effort and dye yield measured in the field and experimental simulations (Michel-Morfín and Chávez 2000, Michel-Morfín *et al.* 2002b).

Model development

The model relied on a description of age groups by sex, where changes in numbers over time caused by natural and fishing mortality were described by difference equations. The analysis was based upon fitting data of age groups or cohorts of the von Bertalanffy growth equation (vBGE):

$$l_t = L_\infty[1 - e^{-K\,(t-t_0)}]$$

Where:

l_t = Conch length at time t
L_∞ = Asymptotic conch length
t_0 = Theoretical age at length = 0
K = Growth coefficient (per year)

A power regression allowing transformation of shell length (L) into weight W ($W = a\,L^b$) was also used, where *a* and *b* were coefficients (condition factor and coefficient of allometry, respectively) specific for each sex of the purple conch. Parameter values used to describe the stock dynamics are shown in Table 1 (after Michel-Morfín *et al.* 2000).

Annual age groups were considered. The equation describing survival of purple conch over time was as follows:

$$N_t = N_o\, e^{-zt}$$

Where:

N_o = Number of individuals of an age group at initial time
N_t = Number of individuals of an age group at time t
Z = Total mortality (M + F)
M = Natural mortality
F = Mortality caused by milking and handling, equivalent to fishing mortality of fisheries models

Natural mortality was defined as one of the Beverton invariants (Jensen 1996, 1997), M = 1.5K. The M values for each

sex (Table 1) were M = 0.405 (females) and M = 0.315 (males). Estimates of fishing mortality as a function of milking frequency were made based on experimental data on survival after milking from Michel-Morfín and Chávez (2000) for the milking period in the model. Incidental mortality equal to 0.0033 per year, presumably caused by trampling and other perturbation factors, was added to the stock when conchs were exploited, even under low exploitation intensities. Longevity, and therefore total number in each age group, was defined under the assumption that animals survive to at least 95% of their asymptotic length, and then by making the appropriate substitution in the vBGE, where the variable t was left as unknown. Thus an estimate of longevity (t_T) was obtained as $t_T = 3/K$, a transformation of the von Bertalanffy growth model formed by making l = 0.95L in the equation and leaving t (= t_T) as unknown.

Population parameters and the dye production per age group were integrated in a model where simulations were made for 25-year periods so that different management scenarios could be tested. Population size referred to mean density of purple conch observed in the field at the intertidal rocky shoreline where these animals occur in bands 2 m wide by 50 km long, and assuming homogeneous density. Extrapolation to different stock sizes was made by multiplying observed mean densities by the length of coastline.

Reproductive contribution was estimated based on age of sexual maturity, fecundity per female, and sex ratio. The purple conch spawns once a year, during the spring (Michel-Morfín *et al.* 2002a). This information allowed estimation of recruitment rate (Michel-Morfín *et al.* 2000), for simulation the model of Beverton and Holt (1957) was used:

$$R = (R_{max} * E) / (E + bR_{max})$$

Where:

R = Number of one-year old recruits produced by the reproduction of adult stock one in the previous year
R_{max} = Maximum number of recruits
E = Parameter of the model related to the product of the number of females times the mean egg production per female
b = Parameter of the model

One-year-old conchs were considered as recruits. Number of recruits was determined for sampling data once adult number was known and assuming a relatively stable stock, such that the difference between adult stock size and the estimated number of specimens per age group was minimized. Parameter values used by the model are shown in Table 1. Random variation of recruitment caused by climate was simulated by multiplying the estimate of recruit number each year by a coefficient of variation that varied randomly and ranged ± 20%. This variation exceeds natural variability. Once recruitment number was determined for each year, this number was multiplied by the sex ratio determined from data from field sampling. Age of sexual maturity is two years.

Estimates of the numbers of males and females of each cohort were made over time. Minimum legal size for milking is 30 mm total shell length, equivalent to an age of two years. Observed density of exploited age groups was 0.86 m^{-2} ± 0.013 (mean ± se) (Michel-Morfín *et al.* 2002b). A power regression ($V = aL^b$) allowed estimating the volume V of dye produced per conch for different lengths L, where a = 0.001 or 0.0005, and b = 2.43 or 2.06, for females and males, respectively (Michel-Morfín *et al.* 2000).

Economic data and tuning up the model

There are no historical records of the number of snails previously exploited or the volume of dye produced. Therefore, data published by Michel-Morfín *et al.* (2002b) on volume of dye extracted per person per unit area and time were used. In addition, data published by Turok *et al.* (1988), Turok (1996), and information from interviews of people in the study area involved in dyeing skeins and weaving them served as complementary sources of information.

Total volume of dye harvested was determined by multiplying the mean volume of dye milked per age group in the proportions occurring at the shore and multiplying this by the mean number of conchs that one person can milk per day. A catchability coefficient, proportional to conch density and time invested per person, was determined based on available data for the number of conchs that each person can milk per area and time and the proportion of this number with respect to stock size in that area. From these data it was possible to determine the frequency that each purple conch could be milked. From experimental data (Michel-Morfín and Chávez 2000) on mortality induced by milking size and milking frequency, some parameters useful for management were evaluated and used as a reference in the model as constraints of exploitation to avoid fishing mortality. The exploitable stock size was assumed to be rather constant, depending only on the recruitment success, and only influenced by natural variability.

Variables that could be modified in the model were the number of persons and milking frequency in the three-month exploitation period per year. The number of milking months considered as a reference was three (October through December) because the reproductive period of purple conch occurs from January to May, followed by the hurricane season from June to September. These factors constrain exploitation. The model allowed estimation of total volume of dye and the economic benefits under different scenarios, such as the number of persons and milking frequency.

Mean density of exploited stock, volume of dye produced, fishing mortality, benefit / cost ratio, and number of stained skeins were state variables defined for each year of simulation. Each stained skein requires 921 purple conchs to be milked, producing 625 ml of dye; each fisherman can collect approximately 2.5 L per week from 1250 m of coastline length (Michel-Morfín *et al.* 2002b). For planning purposes, 50 km of shoreline (in a 2 m-wide band of 100,000 m^2) were arbitrarily used as stock units in a three-month fishing season; results refer to these units.

Economic benefits for this activity were obtained from data on the value of dye over time (Table 1). Costs were determined by considering the price of fuel consumed by an outboard motor per day ($25.00 USD) and a salary equivalent to minimum wage ($100.00 USD/month). The amount of dye required to stain a cotton skein allowed estimation of the number of skeins to be stained with the amount of dye produced over a certain length of shoreline and for a given exploitation intensity, *i.e.* the number of fishermen and milking frequency (7, 14, 21, or 28 days). Once certain conditions defining an exploitation scenario were chosen, simulations were made for 30 years, and initial conditions were maintained. This way biological and socio-economic consequences could be evaluated at the end of the simulation, allowing comparison of each scenario and consequently defining and optimizing different management options.

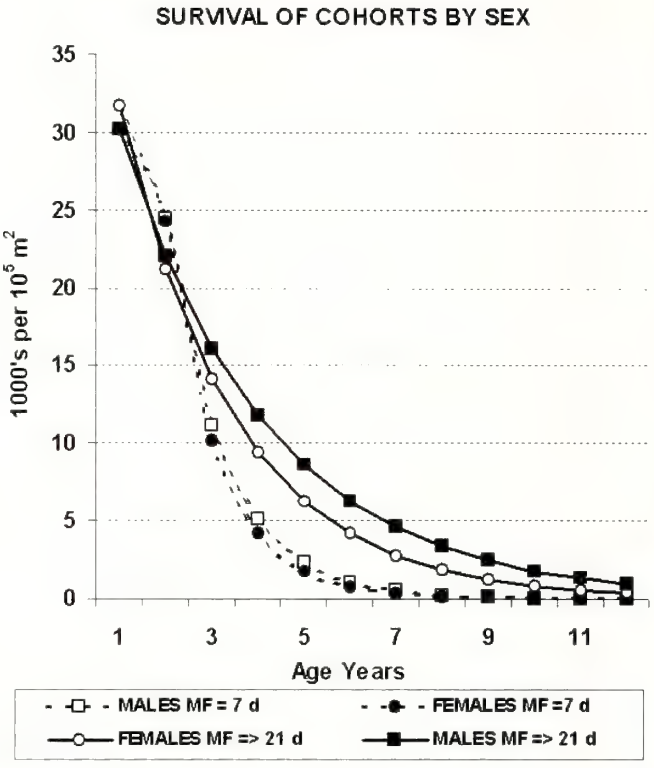

Figure 1. Survival of cohorts of *Plicopurpura pansa*, showing the effects of differential sex ratio (Females F = 47%, Males M = 53%) and differential growth rates (von Bertalanffy growth coefficients: $K_F = 0.27$, $K_M = 0.21$). When the stock of purple conchs is milked with a frequency of ≥21 days, there is no fishing mortality. The lower two lines show the effect of milking frequency (MF) of 7 days (d) and 2 years as the age of first milking, or minimum legal size.

RESULTS

Stock dynamics

Recruitment of juvenile conch occurs approximately four months after egg laying. Given the differential sex ratio, growth rates, and natural mortalities of purple conchs, recruitment age to shoreline is four months. At this age the number of recruits in the 50 km by 2 m-wide band is 30,180 males and 31,730 females. However, because growth rate is higher in females, longevity is necessarily shorter, as required to maintain the sex ratio observed in the field (0.47 to 0.53 for females to males, respectively). This is evidenced in Fig. 1, which shows a stock under fishing pressure, with milking frequency ≥21 days, describing survival of a cohort affected by natural mortality only, regardless of the number of persons. By contrast, the effect of intense exploitation (30 fishermen) on milked age groups is also shown in the same figure with a milking frequency of seven days. In this case the impact of dye harvest on survival of exploited age groups was remarkable.

Variables used to define exploitation scenarios were the number of fishermen (between 1 and 60) and the milking frequency (7, 14, 21, and 28 days) over the three-month milking season. Combinations of these variables allowed simulation of many exploitation strategies. Output was total dye volume, stock size, exploitable stock, the benefit/cost ratio, and the number of dyed skeins. These results are shown in Figs. 2 and 3 and are described in the following paragraphs.

Dye production

Volume of dye produced depends on the number of fishermen and the milking frequency. Volume of dye increased with milking frequency up to a maximum and then decreased sharply. With milking frequencies of 7 and 14 days, the maximum volume of dye was 110 and 190 L, under exploitation intensities of 25 and 35 fishers, respectively. By contrast, maximum dye volume approached 310 L produced by milking every 21 and 28 days with 40 and 45 persons, respectively (Fig. 2A).

The number of stained skeins varied in direct proportion to the volume of dye produced (Fig. 2B); therefore, the highest number of stained skeins (about 250 to 260 in the

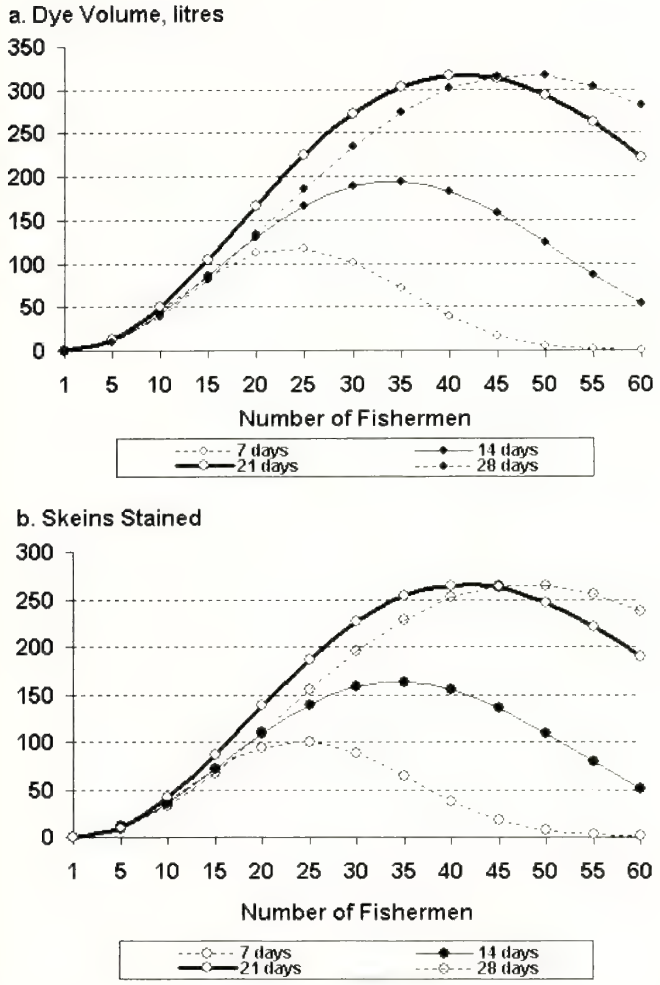

Figure 2. Estimates of dye production from the purple conch, *Plicopurpura pansa*, as a function of the number of fishermen and the milking frequency. A. Volume of dye in liters. B. Number of skeins of cotton thread stained with the dye produced.

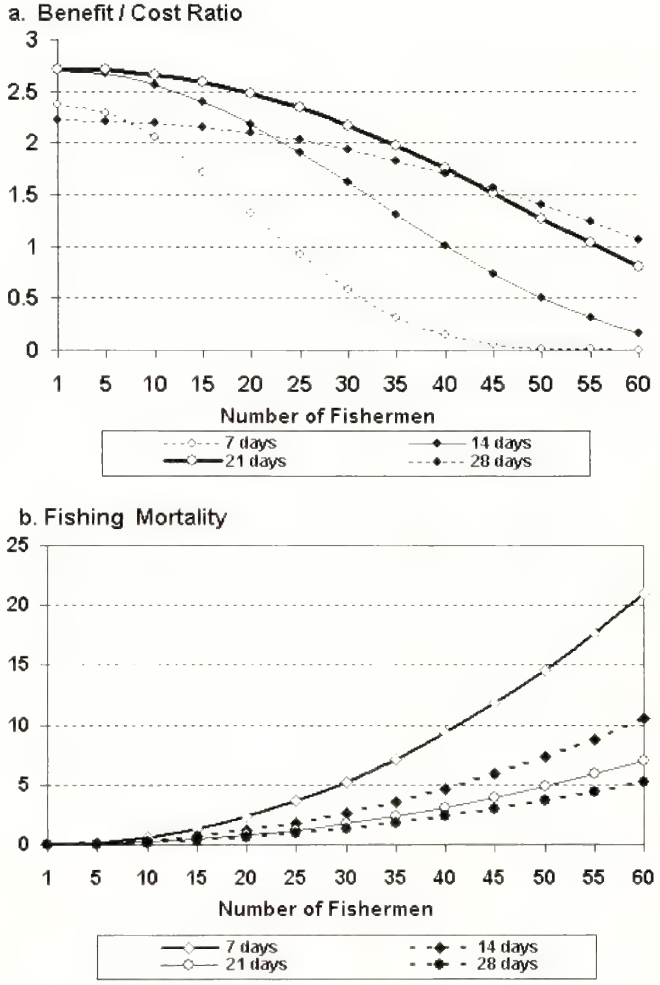

Figure 3. Bioeconomic variables of the dye extracted from the purple conch, *Plicopurpura pansa*, as a function of the number of fishermen and the milking frequency. A. Tendency of the Benefit/Cost ratio. B. Fishing mortality induced by the exploitation intensity.

three-month milking season) could be achieved when purple conchs are milked by 35 to 45 people every 21 days, or by 40 to 55 persons every 28 days.

Impact on the stock and bioeconomic implications

An analysis of benefit to cost (B/C) ratio under the combination of scenarios described above (from 5 to 60 fishers and milking frequencies of 7, 14, 21, and 28 days), yielded a set of four curves (Fig. 3A). The slope was an indicator of the impact of fishing intensity on the stock. A milking frequency of 7 days was clearly a non-viable exploitation scenario, not only because it attained the economic equilibrium level (EEL) when B/C = 1 with only 24 fishers, but also because the impact on the stock mortality was very high. When milking frequency was 14 days, the tendency was about the same, although it took up to 40 persons milking to attain the EEL. In these two scenarios exploitation was not sustainable because the stock could not withstand long-term fishing pressure. A problem with milking every 28 days by 40 to 55 persons is that the B/C ratio was close to the equilibrium level (1.7 to 1.2), which might be risky if the stock was depleted by natural or human-induced factors not accounted for in the model.

The stock was affected by fishing mortality when the frequency of milking was 7 or 14 days, even with low numbers of fishermen (Fig. 3B). In contrast, when milking frequency was $\geq$21 days, fishing mortality was evident only when the number of fishers was >10, approaching F = 0.2 with 50 persons. Dye production reached high levels with

low milking frequencies and high numbers of fishers despite some fishing mortality.

Experimental work (Michel-Morfin and Chávez 2000) showed that milking frequency ≥21 days does not cause mortality. Therefore, any milking frequency ≥21 days represents a suitable exploitation strategy. The economic feasibility of exploiting dye from purple conch showed that profitable activity may be possible under low fishing intensity. The B/C ratio was >2 when milking frequency was 7 days only if the number of persons was <10. When milking frequencies ranging from 14 to 28 days were applied, then B/C > 2 and the fishery could support a fishing intensity of up to 22 persons. The most profitable activity (B/C > 2), with the highest fishing intensity (34 fishers), could be attained when milking frequency was 21 days; when the number of fishermen was lower than 15, B/C > 2.5.

DISCUSSION

Ensuring a sustainable production of dye from purple conch depends on several factors, including milking frequency, which requires temporary closure of areas where intense milking occurs. Exploitable stock size depends on the density of purple conch and the length of coastline to be exploited. In this fishery, each person covers 1250 m of coastline per week (Michel-Morfin *et al.* 2002b). Under the scenario with the highest exploitation intensity, it was likely that the same individual would be milked multiple times over a period of less than 21 days, increasing the probability of fishing mortality. In spite of high values of V_{MF} corresponding to milking frequencies of 7 and 14 days, we suggest that a convenient interval of 21 days be applied to ensure sustainability and to minimize mortality.

Other important constraints to ensure a sustainable exploitation include a minimum shell length of 30 mm, careful handling of conchs during milking, and closing of the fishery for nine months, constrained by the reproductive period from January through May and by the hurricane season from June through September. Minimum exploitation length (30 mm) was based on government regulations to manage the exploitation of purple conch (Anonymous 1988, 1994).

Previous results and those obtained here are part of a long-term study to determine the viability of a sustainable exploitation of the stocks of purple conch. Our findings show that profitable and sustainable exploitation are feasible along the distribution range of the stocks, that is, along the tropical rocky shores of the eastern Pacific, under criteria framed by the principles for sustainable exploitation of living resources (Grumbine 1994, Olver *et al.* 1995).

The parameter values and results of the model developed in this study correspond to the fishery of dye from purple conch along the coasts of the State of Jalisco, in western Central Mexico. Therefore, some variability in parameter values in other portions of the distribution range of the purple conch are likely to be expected. With the aid of GIS mapping we hope to obtain estimates of rocky shores with potential exploitation of dye from purple conch. However, each area should be treated independently and evaluated before opening new zones to exploitation, so that local variability can be measured accurately and evaluated appropriately.

Ensuring that conchs are not milked more than once during the fishing season is critical and should be applied to all fisheries of purple conch. To do this requires restriction of the number of fishermen as well as the areas to be exploited, giving the snails a chance for recovery after being milked. A *modus operandi* similar to that used for the exploitation of the "loco" (*Concholepas concholepas* Bruguière 1789), a gastropod living on the intertidal rocky shore of Chile, could be adopted, in which fishermen are involved in the fishery as well as in surveillance and enforcement of the exploitation zones to avoid poaching. Adoption of this model could allow testing different harvesting regimes because each area would serve as a replicate, allowing evaluation of experimental results by region. Overfishing of the resource would therefore likely be avoided (Castilla and Fernández 1998). Dye production has important implications for regional employment and economic stability. Maximizing dye yield from the purple conch fishery is critical for employing not only the fishers themselves, but for supporting the entire community involved in production of ceremonial clothing.

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LITERATURE CITED

Anonymous. 1988. *Acuerdo Intersecretarial, entre las Secretarías de Pesca, Educación Pública, y Desarrollo Urbano y Ecología, con el que se Regula el Desarrollo, Conservación y Aprovechamiento del*

Caracol Purpura pansa, *Beneficiando a los Núcleos de Población que Tradicionalmente lo han Explotado*. México, D. F. [13 March 1988].

Anonymous. 1994. Norma Oficial Mexicana NOM-059-ECOL-1994, que determina las especies y subespecies de flora y fauna silvestres terrestres y acuáticas en peligro de extinción, amenazadas, raras y las sujetas a protección especial, y que establece especificaciones para su protección. *Diario Oficial de la Federación* [Monday 16 May 1994].

Beverton, R. J. and S. J. Holt. 1957. On the dynamics of exploited fish populations. *U. K. Ministry of Agriculture. Fisheries Investigations* (2)**19**: 1-533.

Castilla, J. C. and M. Fernández. 1998. Small-scale benthic fisheries in Chile: On co-management and sustainable use of benthic invertebrates. *Ecological Applications.* **8**: S124-S132.

Chávez, E. A. 1994. Simulación de la pesquería de sierra (*Scomberomorus maculatus*) del Golfo de México. *Revista de Investigaciones Marinas* **15**: 209-217.

Chávez, E. A. 1996. Simulating fisheries for the assessment of optimum harvesting strategies. *NAGA, The ICLARM Quarterly* [April 1996]: 33-35.

Chávez, E. A. and F. Arreguín-Sánchez. 1994. Simulation modeling for conch fishery management. *In*: R. Appeldoorn and B. Rodríguez, eds., *Queen Conch Biology, Fisheries and Mariculture.* Fundación Científica Los Roques, Caracas. Pp. 125-136.

Cruz-Romero, M., E. A. Chávez, E. Espino and A. García. 1996. Stock assessment of a snapper complex (*Lutjanus* spp.) of the eastern tropical Pacific. *In*: F. Arreguín-Sánchez, J. L. Munro, M. Balgos, and D. Pauly, eds., *Biology, Fisheries and Culture of Tropical Groupers and Snappers.* International Center for Living Aquatic Resources Management, Manila. Pp. 330-336. [ICLARM *Conference Proceedings* No. 48].

Die, D. J. and R. E. Watson. 1992. A per-recruit simulation model for evaluating spatial closures in an Australian penaeid fishery. *Aquatic Living Resources* **5**: 143-153.

Grant, W. E. 1986. *Systems Analysis and Simulation in Wildlife and Fisheries Sciences.* John Wiley and Sons, New York.

Grumbine, R. E. 1994. What is ecosystem management? *Conservation Biology* **8**: 27-38.

Jensen, A. L. 1996. Beverton and Holt life history invariants result from optimal trade-off of reproduction and survival. *Canadian Journal of Fisheries and Aquatic Sciences* **53**: 820-822.

Jensen, A. L. 1997. Origin of the relation between K and L_{inf} and synthesis of relations among life history parameters. *Canadian Journal of Fisheries and Aquatic Sciences* **54**: 987-989.

Keen, A. M. 1971. *Sea Shells of Tropical West America*, 2nd Ed. Stanford University Press. Stanford California.

Michel-Morfín, J. E. and E. A. Chávez. 2000. Effect of repetitive dye extraction over yield and survival rate of the purple conch *Plicopurpura pansa* (Gould, 1853). *Journal of Shellfish Research* **19**: 913-917.

Michel-Morfín, J. E., E. A. Chávez, and V. Landa. 2000. Population parameters and dye yield of the purple conch *Plicopurpura pansa* (Gould, 1853) of West central Mexico. *Journal of Shellfish Research* **19**: 919-925.

Michel-Morfín, J. E., R. Alvarado, and C. Quiñones. 2002a. Fecundidad y morfometría de cápsulas y huevos del caracol del tinte *Plicopurpura pansa* en condiciones de laboratorio. *Boletín del Centro de Investigaciones Biológicas* **36**: 217-230.

Michel-Morfín, J. E., E. A. Chávez, and L. González. 2002b. Population structure, effort and dye yielding of the conch *Plicopurpura pansa* (Gould, 1853) in the Mexican Pacific. *Ciencias Marinas* **28**: 357-368.

Olver, C. H., B. J. Shuter, and C. K. Minns. 1995. Toward a definition of conservation principles for fisheries management. *Canadian Journal of Fisheries and Aquatic Sciences* **52**: 1584-1594.

Prince, J. D. 1992. Using a spatial model to explore the dynamics of an exploited stock of the abalone *Haliotis rubra*. *In*: S. A. Shepherd, M. J. Tegner, and S. A. Guzmán del Proo, eds., *Abalone of the World. Biology, Fisheries and Culture. Proceedings of the 1st International Symposium of Abalone.* Fishing New Books, Oxford. Pp. 305-317.

Ríos-Jara, E., H. León, L. Lizárraga-Chávez, and J. E. Michel-Morfín. 1994. Producción y tiempo de recuperación del tinte de *Plicopurpura patula pansa* (Neogastropoda: Muricidae) en Jalisco, México. *Revista de Biología Tropical* **42**: 537-545.

Turok, M. 1996. Xiuhquilitl, Nocheztli y Tixinda. Tintes del México antiguo. *Arqueología Mexicana* **12**: 26 -33.

Turok , M., A. M. Sigler, E. Hernández, J. Acevedo, R. Lara, and V. Turcott. 1988. *El Caracol Purpura una Tradición Milenaria en Oaxaca.* Dirección General de Culturas Populares, SEP, México D. F.

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The freshwater gastropods of Iowa (1821-1998): Species composition, geographic distributions, and conservation concerns

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Abstract: Although gastropods are important members of freshwater communities, the geographic range, ecological requirements, and conservation status of most species are poorly known. To advance this understanding, I used survey data from museums and peer-reviewed literature to summarize knowledge of the taxonomic composition and geographic distributions of freshwater gastropods in Iowa, U.S.A. Excluding records likely based on erroneous reports, 49 freshwater gastropod taxa (47 species and 2 genera with unknown numbers of species) inhabited Iowa during all or part of the period when records were collected (1821-1998). The Mississippi River and nearby tributaries of eastern Iowa and the prairie pothole and kettlehole regions of northern Iowa historically supported a large number of taxa. In contrast, few gastropods have been reported from the loess soils ecoregion of southwestern Iowa. Although recent improvements in water quality and increases in wetland habitat have likely benefited many gastropod taxa, it appears that as many as 18 species are now imperiled or extirpated from Iowa, and an additional 7 species were much less widespread at the end of the 20th century than formerly. These 25 species of conservation concern were identified on the basis of rarity or absence of recent records and on evidence of local extinctions that were associated with pollution and habitat loss. By comparing data summarized in this review with future data from field surveys, evidence of restricted or shrinking geographic ranges can be provided, and the true conservation status of Iowan gastropods will be determined. This information is of critical importance in establishing legal protection and action plans for the recovery of endangered species.

Key words: biogeography, Gastropoda, endangered species, macroinvertebrates, snails

Gastropods constitute a high percentage of the macroinvertebrate biomass in many freshwater benthic habitats, and their dramatic effects on ecosystem and community dynamics are well documented (Brönmark and Vermaat 1998, Brown 2001). These snails occupy a central position in food webs; they graze on periphyton and detritus, and in turn are consumed by a variety of invertebrate and vertebrate predators (Dillon 2000, Brown 2001). When gastropods are abundant, they regulate benthic primary productivity and algal community structure, and function as important conduits of energy- and nutrient-flow from microorganisms and decomposing organic matter to fish and wildlife (Brönmark and Vermaat 1998, Brown 2001).

Despite ample evidence that freshwater gastropods are ecologically important, we have a poor understanding of the geographic distribution, environmental requirements, and conservation status of most North American species (Neves *et al.* 1997, Bogan 1998). Relative lack of attention to the natural history and taxonomy of freshwater gastropods are key reasons for our weak knowledge base. More than 180 years after Thomas Say published the first descriptions of North American freshwater gastropods, taxonomic confusion still makes it impossible to quantify accurately the geographic ranges and population sizes for many species (Say 1817, Neves *et al.* 1997, Dillon *et al.* 2002). Additionally, federal and state natural resource agencies generally have little interest in freshwater gastropods, and lack of studies of gastropod ecology and geographic distribution is attributed, in large part, to lack of financial support (Neves *et al.* 1997).

Clearly, neglect of freshwater gastropods has proved costly. At least 42 species have become extinct following European settlement of North America (Neves *et al.* 1997, Bogan 1998). Twenty of the remaining 500-600 species are considered by the United States government to be threatened or endangered, and approximately 200 species were recently listed as candidates for inclusion on the federal list of endangered and threatened species (Neves *et al.* 1997, Bogan 1998, U.S. Fish and Wildlife Service 2005). These and other species of freshwater gastropods are thought to have experienced dramatic population declines, but quantitative evidence needed to facilitate legal action and recovery plans are rarely available (Angelo *et al.* 2002, Stewart and Dillon 2004).

Field surveys provide critical evidence of changes in freshwater gastropod assemblages, including population declines and shrinking or restricted geographic ranges. Although large quantities of survey data exist for many North American species, these data are usually scattered among museum collections and literature that can be difficult to obtain. These data must be summarized and disseminated to obtain an accurate assessment of the geographic distribution and conservation status of freshwater gastropods.

I reviewed and summarized data from field surveys in museum databases and peer-reviewed literature to describe

the species composition and geographic distribution of freshwater gastropods in Iowa, U.S.A. Geographic information associated with collection records was used to produce maps and narrative descriptions of distributions of species inhabiting Iowa now or historically. Additionally, I identified species of potential conservation concern on the basis of rarity or absence of recent records and historical evidence of local extinctions. This is the first comprehensive review of knowledge regarding Iowa's freshwater gastropods, and is part of a recent initiative to determine the species composition and conservation status of freshwater gastropods in all North American states and provinces (Freshwater Mollusk Conservation Society 2005).

METHODS

Study area

Iowa is situated in the middle interior of the United States and is considered one of the northern Great Plain states. Iowa extends from 40°35′N to 43°30′N in latitude, from 89°5′W to 96°31′W in longitude, and encloses a total area of 145,003 km^2 (Prior 1991, Tobin 2000). Most of the landscape is characterized by low relief and rolling hills, but the Paleozoic Plateau of northeastern Iowa is a notable exception, consisting of rugged terrain with abundant limestone cliffs (Prior 1991). Elevation in the state ranges from 145-509 m above sea level (Tobin 2000).

Iowa's climate is characterized by hot summers and cold winters. Annual temperatures average 8-11°C, but extremes of −44°C and 48°C have been recorded (Tobin 2000). The state receives an annual average of 81 cm of precipitation. Moderate precipitation, in combination with fertile soils derived from glacial drift and subsequent weathering processes, enabled Iowa to become one of the most productive agricultural regions in the world (Prior 1991, Tobin 2000). Almost 95% of the land surface is now used for farming (Tobin 2000). Farms replaced almost all of the tallgrass-prairie grasslands and forests that dominated the terrestrial landscape at the time of European settlement.

Agriculture and other anthropogenic activities have also impacted Iowa's freshwater ecosystems. Up to 99% of the original 31,000 km^2 of wetlands constituting the prairie pothole complex in northern Iowa were lost to agriculture and development (Bishop *et al.* 1998, Euliss and Mushet 1999). However, wetland habitat is again increasing in Iowa, with at least 240 km^2 of prairie pothole wetlands restored since 1988 (Bishop *et al.* 1998). Similarly, pollution from sewage and industrial effluent has degraded many lakes and rivers, but recent improvements in technology for the treatment of wastewater have had positive effects on water quality in these ecosystems (Shimek 1935a, Bovbjerg *et al.* 1982). Water quality in Iowa's large lakes is of particular concern. These ecosystems are few in number and lakes occurring in the kettlehole region of northcentral and northwestern Iowa provide the only known habitat in Iowa for many species (Shimek 1915, Shimek 1935a). In contrast to large lakes, rivers are abundant. The Mississippi and Missouri River drainages represent Iowa's two major watersheds (Fig. 1A). Rivers in eastern Iowa flow toward the southeast and empty into the Mississippi River, which constitutes the eastern boundary of the state. Western rivers flow toward the southeast to the Missouri River, which represents Iowa's western border (Prior 1991, Tobin 2000).

Study design

Gastropod distributional information was obtained from peer-reviewed literature and museum records that were available as of January 2004. I reviewed any refereed publication that might contain distributional data for freshwater gastropods in Iowa. A total of 910 publications were

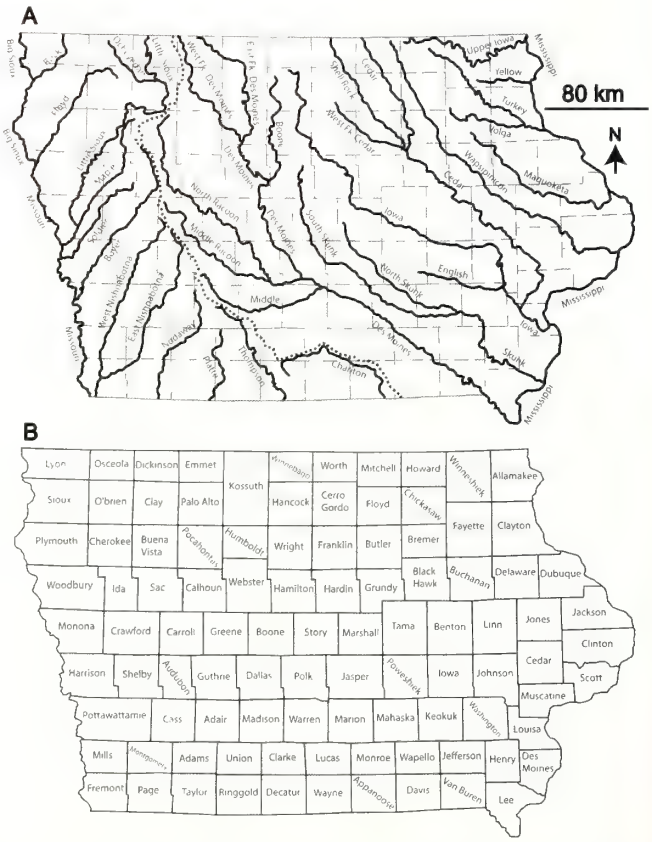

Figure 1. The (A) major rivers and (B) counties of Iowa. The dark dashed line in (A) represents the boundary of the Mississippi River-Missouri River watersheds (maps adapted from Prior 1991 and constructed using Adobe Illustrator® 10 for Windows®)

reviewed; 74 of these contained records of freshwater gastropods from Iowa. Museum records were obtained from curatorial staff or databases available on the World Wide Web. I requested records from 40 North American museums known to have molluscan collections; 13 museums provided electronic records of freshwater gastropods collected in Iowa (Table 1).

A map and/or narrative description of geographic distribution was produced for each taxon recorded from Iowa since living freshwater gastropods were first reported in the 19th century. Counties and specific localities of occurrence were the geographical units used to construct maps (Fig. 1B). Distribution maps using watersheds as basic units might reveal more ecological information than maps using counties. However, specific watershed information was rarely included with historical records, whereas counties and other political units were often reported.

Habitat-use descriptions of taxa are included if this information was provided with records. Evidence of temporal change in distribution and abundance of a taxon is also discussed, along with hypothesized causes provided by investigators observing these changes. Maps were not constructed for species I consider to be erroneously reported from Iowa, but rationale for my decisions are provided. Unless otherwise indicated, taxon names and their authorities are based on Turgeon *et al.* (1998).

RESULTS

Results suggest that 49 freshwater gastropod taxa inhabit Iowa now or occurred there at some time during the period when accessible records were collected (1821-1998). These taxa include 47 species and 2 genera with unknown numbers of species. Additionally, I uncovered records of 6 species that I conclude were reported in error (Table 2).

Family Valvatidae

Valvata bicarinata (Lea, 1841). Individuals of *V. bicarinata* were reported from lentic habitats in southeastern and northwestern Iowa (Fig. 2A; Keyes 1888, Shimek 1890, Walker 1902, 1918, Shimek 1915, Baker 1928, BMNH, FMNH). Population changes for this and several other species have been documented by surveys of Lake Okoboji and nearby habitats of Dickinson County in northwestern Iowa. Shimek (1915) reported *V. bicarinata* from deeper waters of Lake Okoboji, the Gar lakes, and nearby ponds and wetlands. However, it was not found in subsequent surveys of Lake Okoboji that were conducted in 1933-1934, 1954-1959, and 1979 (Shimek 1935a, Bovbjerg and Ulmer 1960, Bovbjerg *et al.* 1982). The probable loss of *V. bicarinata* from Lake Okoboji is consistent with the nearly complete destruction of gastropods in that lake between 1915 and 1933 (Shimek 1915, Shimek 1935a). Shimek (1935a) attributed striking declines in the abundance and diversity of gastropods to the degradation of water quality caused by discharge of sewage into the lake.

Valvata lewisi (Currier, 1868). Shimek (1915) reported that *V. lewisi* formerly occurred in the Okoboji region of Dickinson County in northwestern Iowa, but considered this species to be extinct in that region by 1915. I found no other records of *V. lewisi*.

Table 1. Museums with records of freshwater gastropods from Iowa. Abbreviations are used in the text to indicate sources of records. Data sources were accurate as of February 2005.

Museum	Abbreviation	Data source
J. Ford Bell Museum of Natural History	BMNH	Jonathan Slaght (Freshwater Mollusk Collection Manager)
California Academy of Sciences, San Francisco	CAS	Robert Van Syoc (Senior Collection Manager, Invertebrates)
Cincinnati Museum Center	CMC	Steve Matter (Curator of Zoology)
Cleveland Museum of Natural History	CMNH	Joseph Keiper (Curator of Invertebrate Zoology)
Carnegie Museum of Pittsburgh	CMP	Tim Pearce (Assistant Curator and Head of Mollusk Section)
Delaware Museum of Natural History	DMNH	Kevin Roe (Curator of Mollusks)
Florida Museum of Natural History	FMNH	http://www.flmnh.ufl.edu/natsci/malacology/malacology.htm
Illinois Natural History Survey	INHS	http://www.inhs.uiuc.edu/cbd/collections/mollusk/molluskintro.html
Milwaukee Public Museum	MPM	Joan Jass (Curator of Non-insect Invertebrates)
North Carolina State Museum of Natural Sciences	NCM	Arthur Bogan (Curator of Aquatic Invertebrates)
National Museum of Natural History, Smithsonian	NMNH	Linda Ward (Department of Systematic Biology, Invertebrate Zoology)
Ohio State Museum of Biological Diversity	OSM	Thomas Watters (Curator of Molluscs)
Santa Barbara Museum of Natural History	SBM	Paul Scott (Curator of Malacology)

Table 2. Species reported from Iowa, whose occurrence there between 1821 and 1998 is doubtful.

Species	Records (and references)	Rationale for conclusion
Fontigens nickliniana (Lea, 1838)	Mississippi River, Lee County (Jude 1973, Thompson 1973, Gale 1975)	In a review of *Fontigens*, Hershler *et al.* (1990) reported no legitimate records from Iowa or the upper Mississippi River
Somatogyrus amnicoloides (Walker, 1915)	Eldora in Hardin County and the Cedar River at Cedar Falls, Black Hawk County (FMNH)	Known only from the Ouachita River, Arkansas (Burch 1989)
Elimia semicarinata (Say, 1829)	Mississippi River at Muscatine, Muscatine County (FMNH)	An eastern species occurring as far west as Ohio and Kentucky (Burch 1989)
Pleurocera canaliculata (Say, 1821)	Iowa (CMC)	Although a record occurs from Nebraska, this species appears to be restricted to the Ohio and Wabash watersheds (Burch 1989)
Gyraulus albus (Müller, 1774 in Baker 1928)	Davenport in Scott County, Des Moines in Polk County, Cedar Lake at Cedar Rapids in Linn County, and the Lake Okoboji region of Dickinson County (Keyes 1888, Shimek 1893, 1915)	A European species morphologically similar to *Gyraulus deflectus* (Baker 1928, 1945)
Ancylus fluviatilis (Müller, 1774 in Baker 1928)	Davenport, Scott County (Pratt 1876)	A European species (Baker 1928)

Valvata sincera (Say, 1824). Tryon (1870-1871) reported *V. sincera* from an unspecified location in Iowa. I found no other records for this species.

Valvata tricarinata (Say, 1817). Individuals of *V. tricarinata* have been collected from swamps, ponds, lakes, and rivers across Iowa (Fig. 2B; Say 1821, Haldeman 1840-1845, Binney 1865a, Tryon 1865, Pratt 1876, Witter 1878, Shimek 1890, 1915, 1935a, Bardach *et al.* 1951, Bovbjerg and Ulmer 1960, Gale *et al.* 1972, Thompson 1973, Gale 1975, Bovbjerg *et al.* 1982, Coleman 1984, BMNH, FMNH). Historical population trends of *V. tricarinata* in Lake Okoboji mirror the decline and subsequent recovery of water quality in Okoboji and other large northwestern Iowa lakes following improvements in sewage treatment (Bovbjerg *et al.* 1982). Shimek (1915) originally reported *V. tricarinata* to be abundant in both shallow and deep waters of Lake Okoboji, but by 1933-1934 this species was not found in that lake (Shimek 1935a). By the 1950s, *V. tricarinata* was again increasing in abundance in Lake Okoboji, but did not occur at depths exceeding 10 m (Bovbjerg and Ulmer 1960). By 1979, *V. tricarinata* was abundant and had recolonized depths exceeding 20 m (Gale *et al.* 1972, Bovbjerg *et al.* 1982).

Family Viviparidae

Viviparus georgianus (=*Vivipara contectoides*, *Viviparus contectoides*) (Lea, 1834). Individuals of *V. georgianus* are known only from Lee County in extreme southeastern Iowa. This species was recently reported from a lagoon near Montrose and multiple locations in the Mississippi River from Keokuk to 5 km north of Fort Madison (Fig. 2C; Thompson 1973, Gale 1975, FMNH).

Viviparus intertextus (=*Vivipara intertexta*) (Say, 1829). *Viviparus intertextus* is also restricted to the Mississippi River watershed. It has been recorded from slow-flowing areas of the Cedar and Mississippi Rivers and from bayous and sloughs in Lee and Muscatine counties (Fig. 2D; Tryon 1863, 1865, 1870-1871, Binney 1865a, Pratt 1876, Witter 1878, Baker 1905, 1928, Clench and Fuller 1965, Carlson 1968, FMNH, OSM).

Viviparus subpurpureus (=*Vivipara subpurpurea*) (Say, 1829). This viviparid has been reported from several locations within the Mississippi River, where it occurs in rather swift water and inhabits hard substrates (Fig. 2E; Pratt 1876, Shimek 1890, Call 1894, Baker 1905, 1928, Clench and Fuller 1965, FMNH, INHS).

Bellamya chinensis (=*Viviparus chinensis*, *Viviparus malleatus*) (Reeve, 1863). Formerly known as *Cipangopaludina chinensis*, this Asian snail was first reported from Iowa in the mid-1970s from Riverview Park Lagoon, near the Des Moines River in Des Moines, Polk County (Barnhart 1978, Smith 2000). Jokinen (1982) also reported it from an unspecified location in Des Moines.

Campeloma spp. (=*Campeloma coarctatum*, *Campeloma crassulum*, *Campeloma decisa*, *Campeloma decisum*, *Campeloma exilis*, *Campeloma integra*, *Campeloma integrum*, *Campeloma milesi*, *Campeloma milesii*, *Campeloma obesum*, *Campeloma ponderosum*, *Campeloma regulare*, *Campeloma rufum*, *Campeloma subsolida*, *Campeloma subsolidum*, *Cam-*

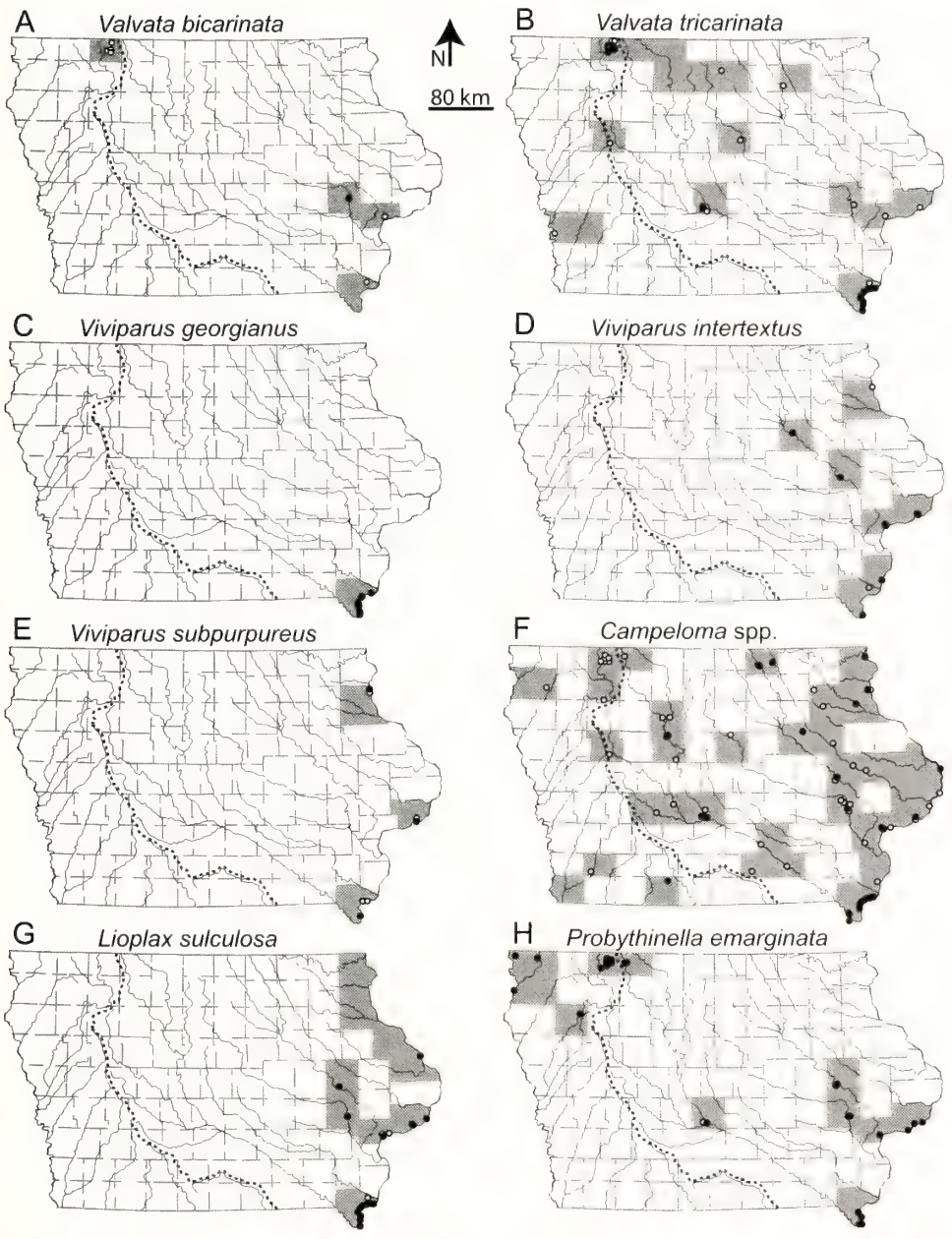

Figure 2. Distributions of (A) *Valvata bicarinata*, (B) *Valvata tricarinata*, (C) *Viviparus georgianus*, (D) *Viviparus intertextus*, (E) *Viviparus subpurpureus*, (F) *Campeloma* spp., (G) *Lioplax sulculosa*, and (H) *Probythinella emarginata* in Iowa. Shading indicates counties where the taxon has been found. Specific localities of occurrence, if known, are indicated by symbols. Unfilled circles indicate records collected before 1950. Filled circles represent records collected during or after 1950, or records from an unknown date.

peloma subsolidus, *Melantho decisa*, *Melantho integra*, *Melantho nolani*, *Melantho ponderosa*, *Melantho subsolida*, *Paludina decisa*, *Paludina integra*, *Paludina ponderosa*, *Paludina regularis*, *Vivipara decisa*, *Vivipara ponderosa*, *Vivipara subsolida*) (Say, 1817). Because of disagreement regarding the number of species in this genus, all *Campeloma* records were mapped together (Shimek 1890, Baker 1928). Individuals of *Campeloma* spp. have been collected from rivers, streams, ponds, and lakes in the Missouri and Mississippi watersheds (Fig. 2F; Haldeman 1840-1845, Tryon 1863, 1865, 1870-1871, Binney 1865a, Pratt 1876, Witter 1878, Call 1880, 1886, 1894, Keyes 1888, Shimek 1890, 1893, 1915, 1935a, Baker 1905, 1928, Van Cleave and Chambers 1935, Carlson 1968, Thompson 1973, Gale 1975, Eckblad *et al.* 1977, BMNH, CMNH, FMNH, NMNH, OSM). Results from surveys of Lake Okoboji suggest that individuals of this genus once occurred in that lake, but were extirpated by 1933 (Shimek 1915, 1935a, Bovbjerg and Ulmer 1960, Bovbjerg *et al.* 1982). However, several recent records exist from elsewhere in Iowa (Carlson 1968, Thompson 1973, Gale 1975, Eckblad *et al.* 1977).

Lioplax sulculosa (Menke, 1827). Although morphologi-

cally similar to *Lioplax subcarinata* (Say, 1816) of the Atlantic drainage in eastern North America, *L. sulculosa* is the only species of *Lioplax* from the Mississippi drainage, and I assigned all records of *Lioplax* to *L. sulculosa* (Clench and Turner 1955). *Lioplax sulculosa* has often been collected from soft substrates in the Mississippi River and nearby tributaries (Fig. 2G; Tryon 1865, Pratt 1876, Witter 1878, Keyes 1888, Van Cleave and Chambers 1935, Clench and Turner 1955, Carlson 1968, Thompson 1973, Gale 1975, Hubert *et al.* 1984, FMNH, OSM).

Family Hydrobiidae

Probythinella emarginata (=*Amnicola binneyana*, *Bithynella obtuse*, *Bythinella obtusa*, *Cincinnatia emarginata*, *Probythinella lacustris*) (Küster, 1852). Individuals of *P. emarginata* have been recorded from ponds, lakes, and rivers in southeastern, central, and northwestern Iowa (Fig. 2H; Witter 1878, Keyes 1888, Shimek 1915, 1935a, Carlson 1968, Bovbjerg *et al.* 1982, Hershler 1996, BMNH, CMC, FMNH). Surveys documented a dramatic decline and recovery of this species in Lake Okoboji during the 20th century. Shimek (1915) first reported it to be common in lakes of the Okoboji region, but it was absent in Lake Okoboji by 1933 (Shimek 1935a). Bovbjerg and Ulmer (1960) also did not find *P. emarginata* in Lake Okoboji from 1954-1959, but Bovbjerg *et al.* (1982) reported it from several locations in that lake in 1979.

Somatogyrus depressus (=*Amnicola depressa*, *Somatogyra depressa*, *Somatogyrus integer*) (Tryon, 1862). This species was first described from snails collected in the Mississippi River at Davenport, Iowa (Tryon 1863). In Iowa, *S. depressus* has been collected from streams and rivers in both Missouri and Mississippi River watersheds (Fig. 3A; Tryon 1863, 1865, 1870-1871, Binney 1865a, Stimpson 1865, Pratt 1876, Keyes 1888, Shimek 1890, 1915, Baker 1928, Carlson 1968, Thompson 1984, Burch 1989, BMNH, CMNH, CMP, DMNH, FMNH, NCM, NMNH).

Birgella subglobosus (=*Birgella subglobosa*, *Somatogyra isogona*, *Somatogyrus isogonus*, *Somatogyrus subglobosus*) (Say, 1825). Individuals of *B. subglobosus* were reported from standing and slow-flowing waters of the Mississippi watershed, including the Mississippi River in southern Iowa, tributaries, and nearby ponds (Fig. 3B; Tryon 1865, Pratt 1876, Witter 1878, Carlson 1968, Thompson 1973, 1984, Gale 1975, BMNH, CMP, FMNH).

Cincinnatia integra (=*Amnicola cincinnatiensis*, *Cincinnatia cincinnatiensis*, *Cincinnatia judayi*) (Say, 1821). The species *C. integra* has a broad historical distribution in Iowa, and is known from ponds, lakes, and rivers in Missouri and Mississippi watersheds (Fig. 3C; Tryon 1865, Pratt 1876, Witter 1878, Keyes 1888, Shimek 1915, 1935a, Hershler and Thompson 1996, BMNH, CMP, FMNH). *Cincinnatia integra* is another species that suffered population declines in Lake Okoboji in the early 20th century. Shimek (1915) considered it to be common in lakes and ponds of the Okoboji region in 1913, but Shimek (1935a), Bovbjerg and Ulmer (1960), and Bovbjerg *et al.* (1982) did not observe individuals of *C. integra* in subsequent surveys of Lake Okoboji. Additional records exist from Lake Okoboji, but dates of these records are unknown (Hershler and Thompson 1996, FMNH).

Pyrgulopsis lustrica (=*Amnicola lustrica*, *Marstonia lustrica*) (Pilsbry, 1890). The species *P. lustrica* has been reported from the Mississippi River in southeastern Iowa and from lakes, streams, and ponds of the Okoboji region in Dickinson County, northwestern Iowa (Fig. 3D; Shimek 1935a, Bovbjerg and Ulmer 1960, Gale 1975, Thompson 1977, Bovbjerg *et al.* 1982, FMNH, NMNH). Shimek (1935a) did not find individuals of *P. lustrica* in his 1933-1934 survey of Lake Okoboji, but stated that the species formerly occurred there. However, *P. lustrica* apparently recovered and was found at several locations in Lake Okoboji in 1954-1959 and 1979 (Bovbjerg and Ulmer 1960, Bovbjerg *et al.* 1982).

Amnicola limosus (=*Amnicola limosa*, *Amnicola orbiculata*, *Amnicola pallida*, *Amnicola parva*, *Amnicola porata*) (Say, 1817). The species *A. limosus* is known from rivers, ponds, and lakes in northern, central, and southeastern Iowa (Fig. 3E; Pratt 1876, Witter 1878, Keyes 1888, Pilsbry 1898, Shimek 1915, 1935a, Bovbjerg and Ulmer 1960, Clampitt 1960, Bovbjerg *et al.* 1982, BMNH, CMNH, CMP, FMNH, NCM). This species also suffered declines and possible extinction in Lake Okoboji early in the 20th century, but was again abundant there in 1979 (Shimek 1915, 1935a, Bovbjerg *et al.* 1982).

Family Pomatiopsidae

Pomatiopsis cincinnatiensis (=*Amnicola sayana*) (Lea, 1840). The species *P. cincinnatiensis* has been reported from four counties in Iowa (Fig. 3F; Tryon 1865, Pratt 1876, Shimek 1915, van der Schalie and Dundee 1955, Gale 1975, Burch and Van Devender 1980). However, records from two counties are questionable. This species is described as semiaquatic; its habitat consists of moist riverbanks (Baker 1928). Although Baker (1928) considered *P. cincinnatiensis* and *A. sayana* to be synonyms, reports of *A. sayana* from the Mississippi River in Lee County in southeastern Iowa and from lakes and ponds of Dickinson County in northwestern Iowa are inconsistent with the habitat requirements of *P. cincinnatiensis* (Shimek 1915, Baker 1928, Gale 1975).

Pomatiopsis lapidaria (Say, 1817). The species *P. lapidaria* is also semiaquatic (van der Schalie and Dundee 1955). It has been reported from moist habitats adjacent to several rivers, but few reports are recent (Fig. 3G; Tryon 1865, Pratt

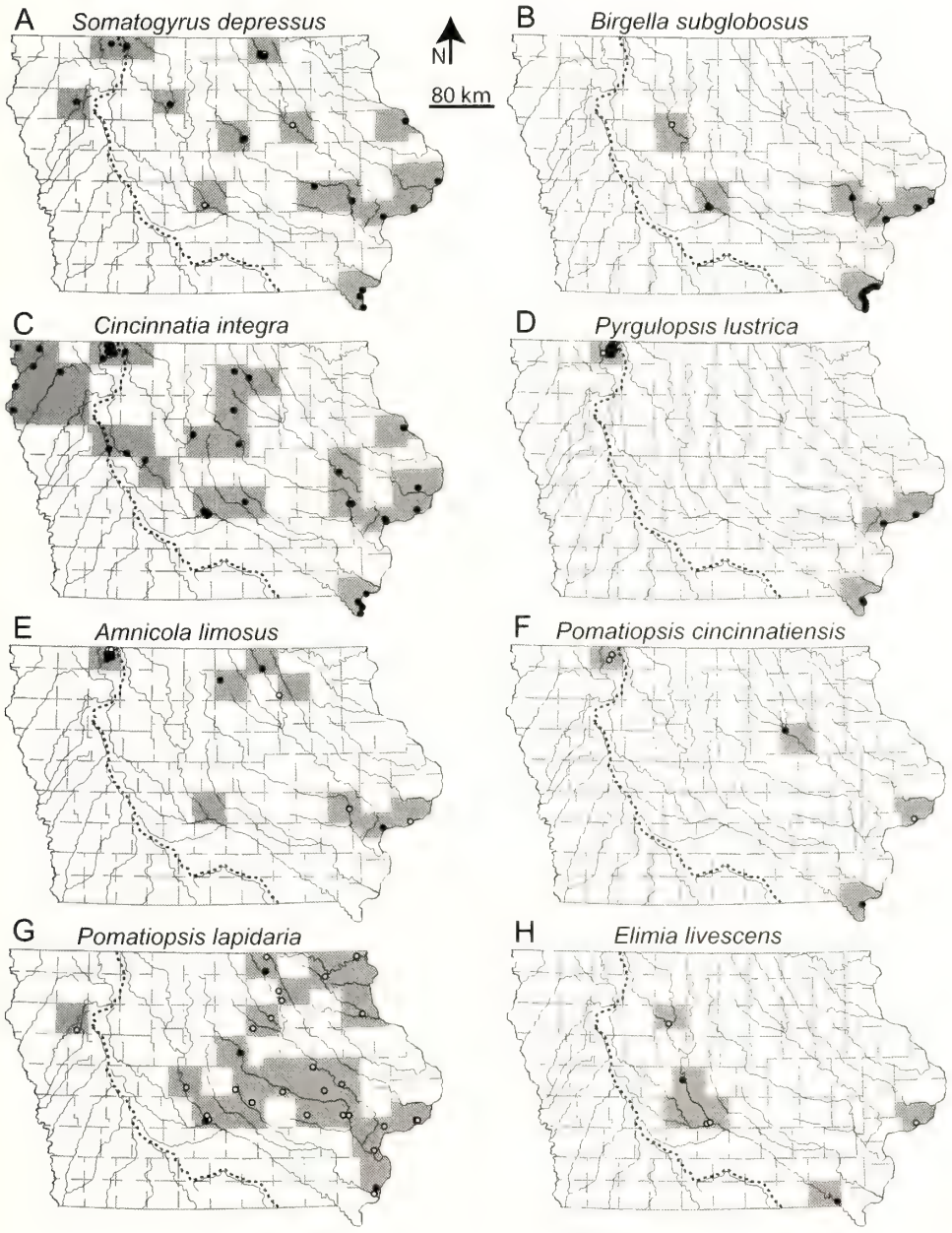

Figure 3. Distributions of (A) *Somatogyrus depressus*, (B) *Birgella subglobosus*, (C) *Cincinnatia integra*, (D) *Pyrgulopsis lustrica*, (E) *Amnicola limosus*, (F) *Pomatiopsis cincinnatiensis*, (G) *Pomatiopsis lapidaria*, and (H) *Elimia livescens* in Iowa. Shading indicates counties where the taxon has been found. Specific localities of occurrence, if known, are indicated by symbols. Unfilled circles indicate records collected before 1950. Filled circles represent records collected during or after 1950, or records from an unknown date.

1876, Pilsbry 1886, Keyes 1888, Shimek 1890, Abbott 1948, Dundee 1957, BMNH, FMNH).

Family Pleuroceridae

Elimia livescens (=*Goniobasis cubicoides*, *Goniobasis cubucoides*, *Goniobasis livescens*) (Menke, 1830). The species *E. livescens* was not often reported, but is known from permanent streams and rivers of the Mississippi watershed (Fig. 3H; Tryon 1865, Pratt 1876, Call 1882, Keyes 1888, Shimek 1893, Dazo 1965, FMNH). This species occurs in habitats dominated by rocks or sand (Dazo 1965).

Pleurocera acuta (=*Pleurocera subulare*, *Trypanostoma subulare*) (Rafinesque, 1831). This species was reported from several rivers in the Mississippi watershed (Fig. 4A; Tryon 1865, Pratt 1876, Witter 1878, Call 1882, Simpson 1895, Dazo 1965, Carlson 1968, Thompson 1973, Gale 1975, BMNH, CMNH, FMNH, INHS, MPM, OSM). Individuals of *P. acuta* occur most frequently in shallow areas of large rivers (Dazo 1965).

Family Lymnaeidae

Acella haldemani (Binney, 1867). Baker (1911) included

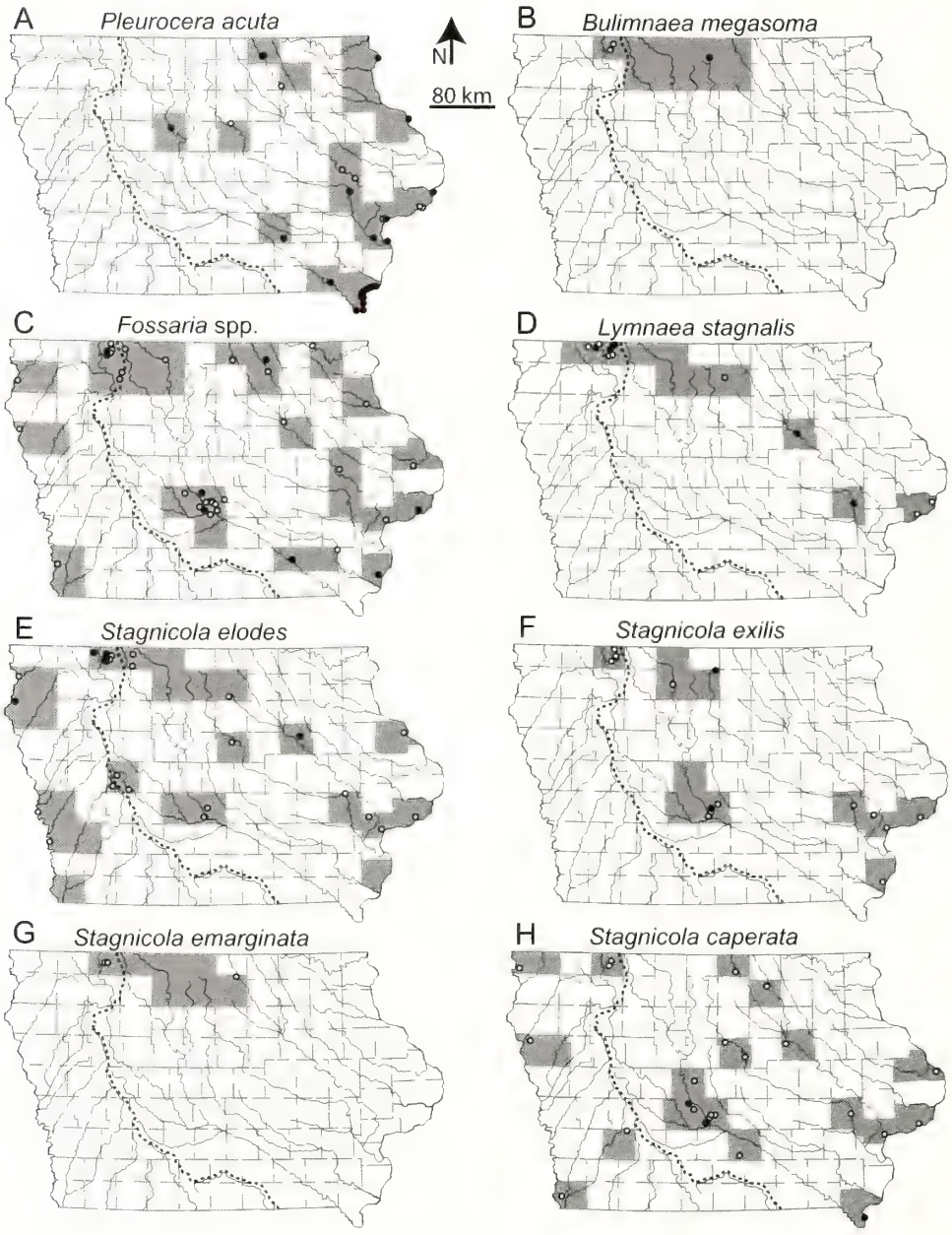

Figure 4. Distributions of (A) *Pleurocera acuta*, (B) *Bulimnaea megasoma*, (C) *Fossaria* spp., (D) *Lymnaea stagnalis*, (E) *Stagnicola elodes*, (F) *Stagnicola exilis*, (G) *Stagnicola emarginata*, and (H) *Stagnicola caperata* in Iowa. Shading indicates counties where the taxon has been found. Specific localities of occurrence, if known, are indicated by symbols. Unfilled circles indicate records collected before 1950. Filled circles represent records collected during or after 1950, or records from an unknown date.

northeastern Iowa as part of the geographic range for *A. haldemani*. However, I found no specific locality records for this species in the state.

Bulimnaea megasoma (=*Bulimnea megasoma*, *Limnaea megasoma*, *Lymnaea megasoma*) (Say, 1824). The species *B. megasoma* was once abundant in lakes and large ponds of northwestern and northcentral Iowa, but an undated report from Winnebago County in northcentral Iowa is the only record that might be recent (Fig. 4B; Keyes 1888, Baker 1911, Shimek 1915, DMNH). Shimek (1915) considered this species to be extinct in the Lake Okoboji region by the early 20th century.

Fossaria spp. (=*Fossaria bulimoides*, *Fossaria exigua*, *Fossaria humilis*, *Fossaria modicella*, *Fossaria obrussa*, *Fossaria parva*, *Galba bulimoides*, *Galba dalli*, *Galba galbana*, *Galba humilis*, *Galba obrussa*, *Galba pallida*, *Galba parva*, *Limnaea decidiosa*, *Limnaea desidiosa*, *Limnaea humilis*, *Limnophysa desidiosa*, *Limnophysa humilis*, *Limnophysa pallida*, *Lymnaea dalli*, *Lymnaea humilis*, *Lymnaea modicella*, *Lymnaea obrussa*, *Lymnaea pallida*, *Lymnaea parva*) (Say, 1822). Taxonomy of the genus *Fossaria* is in a confused state, with species distinguished by minor differences in shell attributes that might be ecophenotypic in origin (Stewart and Dillon 2004). For this reason, I mapped all records of the genus together. This

semiaquatic taxon was collected from exposed mud or shallow submerged areas of ponds, lakes, rivers, and streams throughout Iowa (Fig. 4C; Pratt 1876, Witter 1878, Call 1880, Keyes 1888, Shimek 1890, 1915, Baker 1911, 1928, Bovbjerg and Ulmer 1960, Clampitt 1960, Bovbjerg *et al.* 1982, Coleman 1984, CMNH, CMP, DMNH, FMNH, NCM). The genus *Fossaria* declined in abundance in Lake Okoboji in the early 20th century, but later increased in abundance following improvements in water quality (Shimek 1915, 1935a, Bovbjerg and Ulmer 1960, Bovbjerg *et al.* 1982).

Lymnaea stagnalis (=*Limnaea stagnalis*) (Linnaeus, 1758). The species *L. stagnalis* has been recorded from lakes, ponds, and swamps in northern and eastern Iowa (Fig. 4D; Tryon 1865, Keyes 1888, Baker 1911, Shimek 1915, 1935a, Bovbjerg 1968, Brown 1979a, 1979b, 1983, Coleman 1984, Kessel and Beams 1984, DMNH, FMNH). Keyes (1888) and Shimek (1890) considered *L. stagnalis* to be abundant in northern Iowa. However, Shimek (1915) later remarked that it was no longer common in Lake Okoboji or elsewhere in the region, and neither Shimek (1935a), Bovbjerg and Ulmer (1960), or Bovbjerg *et al.* (1982) reported this species in subsequent surveys of Lake Okoboji. Shimek (1935a) also recognized dramatic declines in abundance of *L. stagnalis* in the entire region of northern Iowa between Spirit Lake in Dickinson County and Clear Lake in Cerro Gordo County. However, recent records suggest this species persisted in wetlands of northern Iowa at the end of the 20th century (Bovbjerg 1968, Brown 1979a, 1979b, 1983, Coleman 1984).

Stagnicola elodes (=*Galba elodes*, *Galba iowaensis*, *Galba palustris*, *Galba reflexa*, *Galba umbrosa*, *Limnaea palustris*, *Limnaea reflexa*, *Limnaea umbrosa*, *Limnaeus elodes*, *Limnea umbrosa*, *Limneus elongatus*, *Limnophysa nuttaliana*, *Limnophysa palustris*, *Limnophysa reflexa*, *Limnophysa umbrosa*, *Lymnaea elodes*, *Lymnaea palustris*, *Lymnaea reflexa*, *Lymneus elongatus*, *Lymneus umbrosus*, *Stagnicola crystallensis*, *Stagnicola palustris*, *Stagnicola reflexa*, *Stagnicola umbrosa*) (Say, 1821). The species *S. elodes* has been recorded from ephemeral and permanent ponds and swamps and from embayments of lakes throughout Iowa (Fig. 4E; Say 1832, Haldeman 1840-1845, Tryon 1865, Pratt 1876, Witter 1878, Call 1880, Keyes 1888, Baker 1904, 1911, 1928, Shimek 1915, 1935a, Bovbjerg and Ulmer 1960, Bovbjerg 1965, 1968, Brown 1979a, Bovbjerg *et al.* 1982, Coleman 1984, CAS, DMNH, FMNH). Although Shimek (1915, 1935a) reported severe reductions in the abundance of *S. elodes* in Lake Okoboji and northern Iowa generally, populations in Lake Okoboji later recovered (Bovbjerg and Ulmer 1960, Bovbjerg *et al.* 1982).

Stagnicola exilis (=*Galba exilis*, *Galba kirtlandiana*, *Limnaea zebra*, *Limnophysa zebra*, *Lymnaea exilis*, *Lymnaea zebra*) (Lea, 1834). Historically, the species *S. exilis* occurred in ephemeral and permanent lentic habitats in northern, central, and southeastern Iowa, but few recent records exist (Fig. 4F; Tryon 1865, Keyes 1888, Baker 1911, Shimek 1915, Bovbjerg 1968, FMNH). It has not been reported from Lake Okoboji since 90 years ago, when Shimek (1915) noted that *S. exilis* was widely distributed but uncommon.

Stagnicola catascopium (=*Galba catascopium*, *Lymnaea catascopium*) (Say, 1867). The only records I found for *S. catascopium* were from northern Iowa in the vicinities of Ruthven and Charles City, in Palo Alto and Floyd counties, respectively (Baker 1911).

Stagnicola emarginata (=*Galba emarginata*, *Lymnaea emarginata*) (Say, 1821). Historically, individuals of *S. emarginata* inhabited the kettlehole region of northern Iowa, encompassing the area between Lake Okoboji in Dickinson County and Clear Lake in Cerro Gordo County (Fig. 4G; Baker 1911, Shimek 1915, Shimek 1935a). Shimek (1935a) remarked that *S. emarginata* was once abundant in the kettlehole region but had vanished by 1933. This species has not been reported from Iowa in more than 90 years.

Stagnicola caperata (=*Galba caperata*, *Limnaea caperata*, *Limnophysa caperata*, *Lymnaea caperata*, *Lymnaea umbilicata*) (Say, 1829). The species *S. caperata* has been reported from marshes, swamps, vernal ponds, and lake margins throughout Iowa, but few observations were made of this wetland species in recent years (Fig. 4H; Pratt 1876, Witter 1878, Keyes 1888, Baker 1911, Shimek 1915, FMNH).

Family Physidae

Physa skinneri (Taylor, 1954). Burch (1989) considered Iowa to be within the geographic range of *P. skinneri*, but I did not find records confirming this. The species occurs in prairie pothole wetlands of the Dakotas and might exist in similar habitats of northwestern Iowa (Euliss *et al.* 1999).

Physella gyrina (=*Physella ancillaria*, *Physella elliptica*, *Physella hildrethiana*, *Physella lordi*, *Physella oleacea*, *Physella sayi*, *Physella sayii*) (Say, 1821). Results from breeding experiments recently revealed a lack of reproductive isolation among several nominal species of *Physella* (=*Physa*), resulting in *Physella ancillaria* (Say, 1825) and *Physella gyrina* being synonymized (Dillon and Wethington 2004). *Physella gyrina* was originally described by Say (1821) from a population located in southwestern Iowa near Council Bluffs, in Pottawattamie County. It has since been found in almost any habitat supporting freshwater snails (Fig. 5A; Say 1821, Haldeman 1840-1845, Binney 1865b, Tryon 1865, Pratt 1876, Witter 1878, Call 1880, Keyes 1888, Shimek 1890, 1915, 1935a, Baker 1905, 1928, Wurtz 1949, Ulmer and Sommer 1957, Bovbjerg and Ulmer 1960, Clampitt 1960, 1970, Ulmer 1960, Bovbjerg *et al.* 1970, Rausch and Bovbjerg 1973, Gale 1975, Te 1975, Brown 1979a, Bovbjerg *et al.* 1982, Coleman 1984, Dillon and Wethington 2004, CMNH, DMNH,

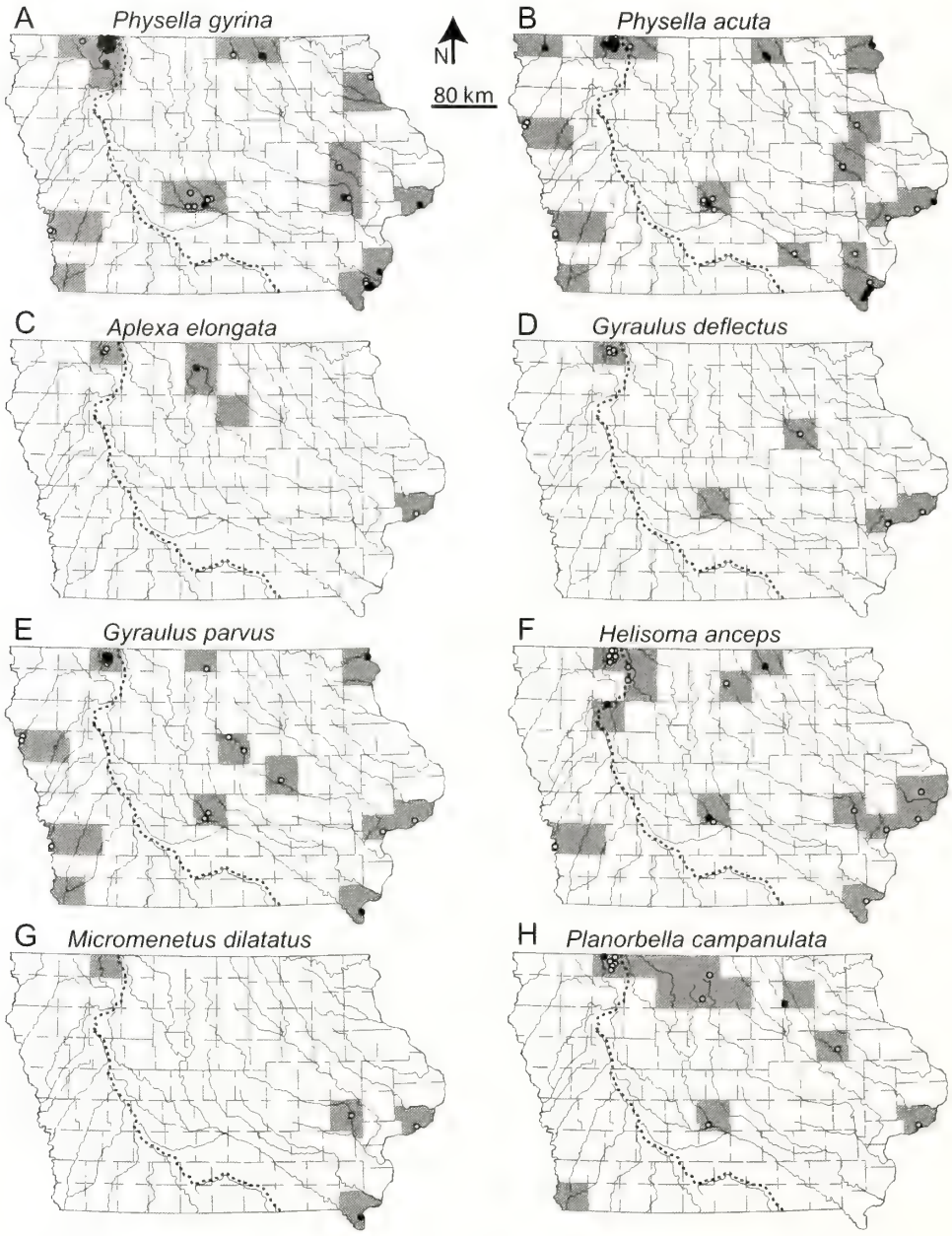

Figure 5. Distributions of (A) *Physella gyrina*, (B) *Physella acuta*, (C) *Aplexa elongata*, (D) *Gyraulus deflectus*, (E) *Gyraulus parvus*, (F) *Helisoma anceps*, (G) *Micromenetus dilatatus*, and (H) *Planorbella campanulata* in Iowa. Shading indicates counties where the taxon has been found. Specific localities of occurrence, if known, are indicated by symbols. Unfilled circles indicate records collected before 1950. Filled circles represent records collected during or after 1950, or records from an unknown date.

FMNH, INHS, MPM, OSM). Similar to other species of gastropods, *P. gyrina* was nearly if not completely extirpated from Lake Okoboji in the early 20th century (Shimek 1915, 1935a). However, *P. gyrina* was very abundant in 1954-1959 and 1979 surveys of Lake Okoboji (Bovbjerg and Ulmer 1960, Bovbjerg *et al.* 1982).

Physella acuta (=*Physella anatina*; *Physella halei*, *Physella heterostropha*, *Physella integra*, *Physella virgata*, *Physella walkeri*) (Draparnaud, 1805). After finding no evidence of reproductive isolation among several species of *Physella* (=*Physa*) reported from Iowa, including *P. acuta*, *Physella heterostropha* (Say, 1817), *Physella integra* (Haldeman, 1841), and *Physella virgata* (Gould, 1855), Dillon *et al.* (2002) assigned the name *Physella acuta* to this entire group. *Physella acuta* has been recorded from almost every freshwater habitat in Iowa (Fig. 5B; Say 1821, Tryon 1865, Pratt 1876, Witter 1878, Call 1880, Keyes 1888, Shimek 1890, 1915, 1935a, Walker 1918, Bovbjerg and Ulmer 1960, Clampitt 1970, Gale 1975, Eckblad *et al.* 1977, Brown 1979a, Bovbjerg *et al.* 1982, Coleman 1984, CMNH, CMP, DMNH, FMNH, OSM). This is one of the most pollution-tolerant freshwater snails, yet even it declined in abundance in Lake Okoboji during the early 20th century (Shimek 1915, 1935a). A few individuals of *P. acuta* were the only gastropods of any kind found in

Lake Okoboji during a 1933-1934 survey (Shimek 1935a). By 1979, this species was again abundant in that lake (Bovbjerg *et al.* 1982).

Aplexa elongata (=*Aplexa hypnorum*) (Say, 1821). Individuals of the species *A. elongata* have been collected from ponds and slow-moving streams in northern and southeastern Iowa (Fig. 5C; Tryon 1865, Pratt 1876, Keyes 1888, Shimek 1890, 1915, Coleman 1984, FMNH). Several malacologists remarked that *A. elongata* was abundant and widely distributed in northern Iowa in the 19th and early 20th centuries (Pratt 1876, Keyes 1888, Shimek 1890, 1915). Shimek (1915) reported *A. elongata* from Lake Okoboji, but it was not found in several subsequent surveys of that lake conducted from 1933-1979 (Shimek 1935a, Bovbjerg and Ulmer 1960, Bovbjerg *et al.* 1982). The scarcity of recent records suggests this species also suffered other local extinctions. However, a recent record from an unspecified location in Dickinson County suggests *A. elongata* still inhabited Iowa near the end of the 20th century (Coleman 1984). An undated record also occurs from Crystal Lake in Hancock County (FMNH).

Family Planorbidae

Gyraulus deflectus (=*Gyraulus hirsutus*, *Planorbis deflectus*) (Say, 1824). Individuals of *G. deflectus* were collected from several swamps, ponds, and lakes, but this species has not been reported from Iowa in almost 60 years (Fig. 5D; Tryon 1865, Pratt 1876, Witter 1878, Keyes 1888, Shimek 1915, 1935a, Baker 1945, FMNH). This species once occurred in shallow areas of Lake Okoboji, but was absent in 1933 and had not returned as of 1979 (Shimek 1915, 1935a, Bovbjerg and Ulmer 1960, Bovbjerg *et al.* 1982).

Gyraulus circumstriatus (Tryon, 1866). An undated report of *G. circumstriatus* from Des Moines in Polk County constitutes the only known record of this species in Iowa (FMNH).

Gyraulus parvus (=*Planorbis parvus*) (Say, 1817). Individuals of *G. parvus* have been found in ponds, swamps, lakes, and slow-flowing vegetated areas of rivers throughout Iowa (Fig. 5E; Say 1821, Tryon 1865, Pratt 1876, Witter 1878, Call 1880, Keyes 1888, Shimek 1915, 1935a, Bovbjerg and Ulmer 1960, Clampitt 1960, Eckblad *et al.* 1977, Bovbjerg *et al.* 1982, Coleman 1984, FMNH). Following a decline in Lake Okoboji in the early 1900s, this species recovered rapidly and was one of the most abundant snails in that lake by 1979 (Shimek 1915, 1935a, Bovbjerg and Ulmer 1960, Clampitt 1960, Bovbjerg *et al.* 1982).

Helisoma anceps (=*Helisoma antrosa*, *Helisoma antrosum*, *Helisoma bicarinatus*, *Planorbis antrosus*, *Planorbis bicarinatus*) (Menke, 1830). This species has been collected from a wide range of habitats throughout Iowa, including ponds, lakes, and rivers (Fig. 5F; Say 1821, Tryon 1865, Pratt 1876, Witter 1878, Keyes 1888, Walker 1909, Shimek 1915, 1935a, Baker 1945, Meierhoff and Prill 1982, Kennedy and Miller 1990, CMNH, DMNH, FMNH, NCM, NMNH). Although recent records exist for *H. anceps* in Iowa, this species apparently disappeared from Lake Okoboji by 1933, and was not observed during subsequent surveys of that lake (Shimek 1915, 1935a, Bovbjerg and Ulmer 1960, Bovbjerg *et al.* 1982).

Micromenetus dilatatus (=*Menetus dilatatus*, *Planorbis dilatatus*) (Gould, 1841). Individuals of *M. dilatatus* have occasionally been collected from lagoons and shallow regions of lakes in Iowa (Fig. 5G; Shimek 1890, 1915, FMNH). A 1969 record from Montrose in Lee County is the only recent record for this species in the state (FMNH).

Planorbella campanulata (=*Helisoma campanulata*, *Helisoma campanulatum*, *Planorbella campanulatum*, *Planorbella campanulatus*, *Planorbis campanulatus*) (Say, 1821). The species *P. campanulata* has been recorded from ponds, lakes, and rivers of the Mississippi and Missouri watersheds (Fig. 5H; Binney 1865b, Tryon 1865, Call 1880, Keyes 1888, Shimek 1915, 1935a, Bovbjerg *et al.* 1970, FMNH). *Planorbella campanulata* was once reported to be common in lakes of northern Iowa (Keyes 1888). Shimek (1915) later remarked that the species was present but uncommon in shallow regions of Lake Okoboji and the Gar lakes in Dickinson County. Individuals of *P. campanulata* were not encountered in more recent surveys of Lake Okoboji (Shimek 1935a, Bovbjerg and Ulmer 1960, Bovbjerg *et al.* 1982).

Planorbella trivolvis (=*Helisoma regularis*, *Helisoma trivolvis*, *Pierosoma trivolvis*, *Planorbis binneyi*, *Planorbis lentus*, *Planorbis trivolvis*) (Say, 1817). This widely distributed species was reported from ponds, wetlands, and quiet areas of rivers (Fig. 6A; Say 1821, Say 1834 in Binney 1858, Binney 1865b, Tryon 1865, Pratt 1876, Witter 1878, Call 1880, Keyes 1888, Shimek 1915, Bovbjerg and Ulmer 1960, Kater and Koneko 1972, Gale 1975, Bovbjerg *et al.* 1982, Coleman 1984, CMNH, DMNH, FMNH, INHS, NMNH, OSM, SBM). *Planorbella trivolvis* was abundant in shallow and swampy areas of Lake Okoboji in the early 20th century, but was not found in a 1933-1934 survey of that lake (Shimek 1915, 1935a). The species was uncommon but present in subsequent surveys of Lake Okoboji (Bovbjerg and Ulmer 1960, Bovbjerg *et al.* 1982).

Planorbella truncata (=*Helisoma truncata*; *Helisoma truncatum*, *Planorbella truncatum*, *Planorbis truncatus*) (Miles, 1861). Individuals of *P. truncata* were recorded from lakes in northern Iowa as late as 1945, but have not been seen anywhere since (Fig. 6B; Shimek 1915, 1935a, Baker 1945, DMNH). Shimek (1915) was the last to report it from Lake Okoboji and the Gar lakes of Dickinson County. Shimek (1935a) also reported *P. truncata* from the kettlehole region between Spirit and Clear lakes in Dickinson and

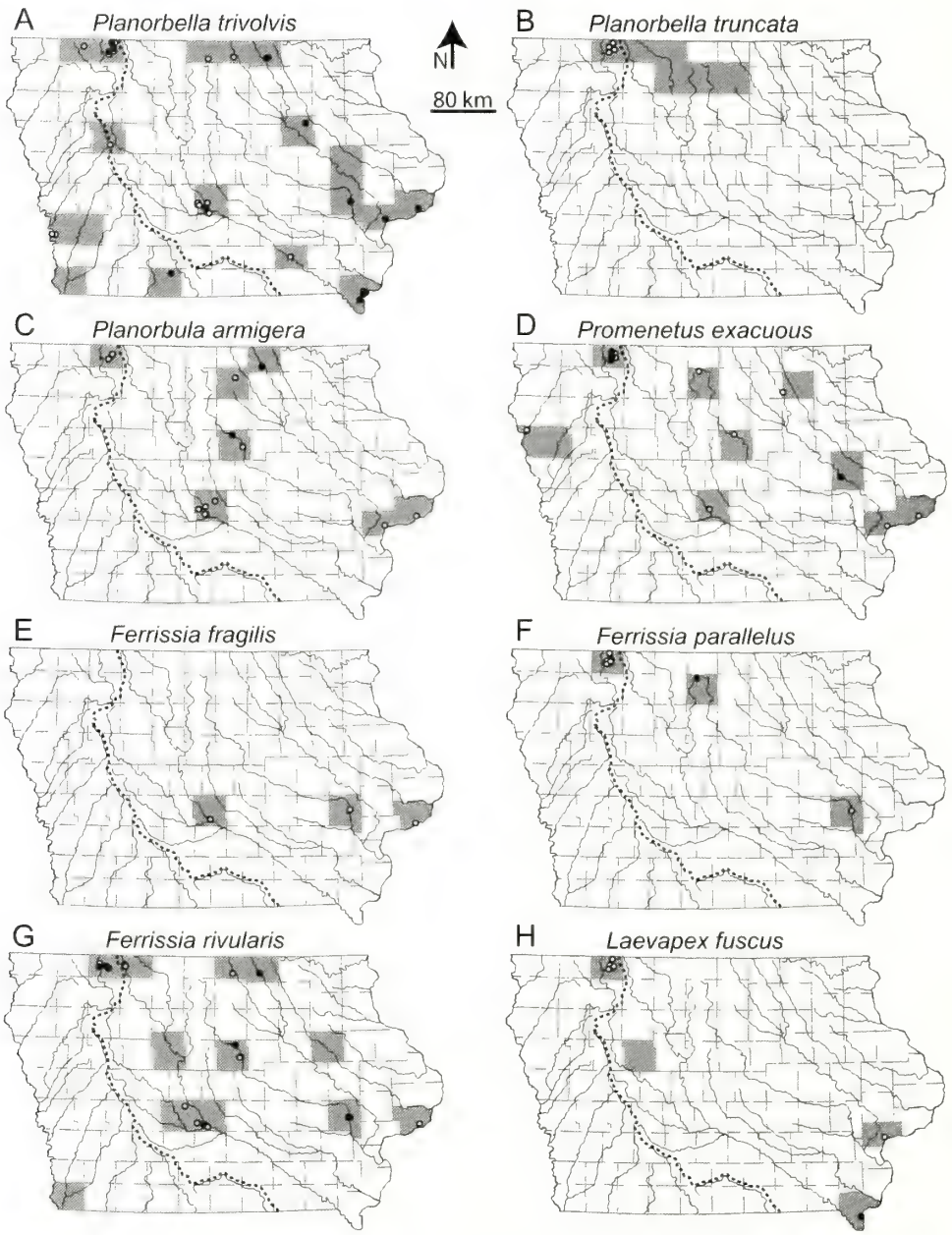

Figure 6. Distributions of (A) *Planorbella trivolvis*, (B) *Planorbella truncata*, (C) *Planorbula armigera*, (D) *Promenetus exacuous*, (E) *Ferrissia fragilis*, (F) *Ferrissia parallelus*, (G) *Ferrissia rivularis*, and (H) *Laevapex fuscus* in Iowa. Shading indicates counties where the taxon has been found. Specific localities of occurrence, if known, are indicated by symbols. Unfilled circles indicate records collected before 1950. Filled circles represent records collected during or after 1950, or records from an unknown date.

Cerro Gordo counties, but remarked that its numbers were much reduced relative to earlier surveys.

Planorbula armigera (=*Planorbella armigera*, *Planorbis armigera*, *Planorbis armigerus*, *Planorbula jenksii*, *Segmentina armigera*, *Segmentina wheatleyi*) (Say, 1821). Individuals of *P. armigera* were reported from several wetlands, lakes, and rivers (Fig. 6C; Tryon 1865, Pratt 1876, Witter 1878, Keyes 1888, Shimek 1915, Baker 1945, CMNH, CMP, DMNH, FMNH). This species was present in shallow regions of Lake Okoboji before 1915, but was not found in later surveys of the lake (Shimek 1915, 1935a, Bovbjerg and Ulmer 1960, Bovbjerg *et al.* 1982). The most recent known sighting in Iowa of *P. armigera* occurred in 1942, although some undated records could be more recent (CMNH, DMNH, FMNH).

Promenetus exacuous (=*Menetus exacuous*, *Menetus exacutus*, *Planorbis exacutus*) (Say, 1821). The species *P. exacuous* has been reported from ponds, lakes, and quiet areas of rivers (Fig. 6D; Tryon 1865, Pratt 1876, Witter 1878, Shimek 1915, 1935a, Baker 1945, Bovbjerg and Ulmer 1960, Clampitt 1960, Bovbjerg *et al.* 1982, Coleman 1984, DMNH, FMNH). Shimek (1915) found individuals of *P. exacuous* in shallow waters of the Lake Okoboji region of Dickinson County in northwestern Iowa. Although he did not report it

from his 1933-1934 survey of Lake Okoboji, it was present at several locations in that lake during 1954-1959 and 1979 surveys (Shimek 1935a, Bovbjerg and Ulmer 1960, Bovbjerg *et al.* 1982).

Promenetus umbilicatellus (=*Gyraulus umbilicatelus*) (Cockerell, 1887). Baker (1945) reported this species from an unspecified location in Iowa. I found no additional records for *P. umbilicatellus.*

Family Ancylidae

Ferrissia fragilis (=*Ancylus pumilus*, *Ferrissia shimekii*, *Gundlachia meekiana*) (Tryon, 1863). Individuals of *F. fragilis* were reported from central and eastern Iowa (Fig. 6E; Pilsbry 1886, Walker 1904, FMNH). This species has not been reported from Iowa since 1912 (FMNH). However, this tiny gastropod is easily overlooked and difficult to distinguish from *Ferrissia rivularis* (Say 1817). *Ferrissia fragilis* is tolerant of organic pollution and might still survive in eutrophic, vegetated wetlands and lakes in Iowa (Basch 1963).

Ferrissia parallelus (=*Ancylus parallelus*, *Ferrissia parallela*) (Haldeman, 1841). *Ferrissia parallelus* was recorded in the 1800s and early 1900s from several lakes in northwestern Iowa, and from a single location in Iowa City, Johnson County (Fig. 6F; Shimek 1890, 1915, 1935a, 1935b, Walker, 1904, FMNH). Shimek (1915) considered this species to be extinct in the Lake Okoboji region of northwestern Iowa by the early 20th century, and with the possible exception of an undated record from Crystal Lake, Hancock County, *F. parallelus* has not been reported from Iowa in more than 80 years (FMNH).

Ferrissia rivularis (=*Ancylus rivularis*, *Ancylus tardus*, *Ferrissia tarda, Ferrissia tardus*) (Say, 1817). The species *F. rivularis* is the most frequently reported ancylid in Iowa (Fig. 6G; Tryon 1865, Pratt 1876, Call 1880, Keyes 1888, Shimek 1915, 1935b, Bovbjerg and Ulmer 1960, Bovbjerg *et al.* 1982, CMNH, FMNH). *Ferrissia rivularis* is considered to be restricted to streams, rivers, and lakes with strong current or wave action (Baker 1928, Basch 1963). Bovbjerg and Ulmer (1960) and Bovbjerg *et al.* (1982) reported this species from a protected bay in Lake Okoboji, but this area is more consistent with the habitat of *Ferrissia fragilis*, a taxon that is morphologically similar to *F. rivularis* (Basch 1963, Burch 1989).

Laevapex diaphanus (=*Ancylus diaphanus*) (Haldeman, 1841). I found two records for *L. diaphanus*, including one from eastern Iowa and one from northwestern Iowa. Specimens were collected from a pond near Iowa City, Johnson County, on an unknown date and from shallow standing waters of the Lake Okoboji region in Dickinson County in the late 1800s and early 1900s (Shimek 1915, FMNH).

Laevapex fuscus (=*Ancylus fuscus*, *Ferrissia fusca*, *Ferrissia kirtlandi*) (Adams, 1841). The species *L. fuscus* has been reported from wetlands, lakes, and rivers in Iowa (Fig. 6H; Witter 1878, Shimek 1935a, 1935b, Gale 1975, FMNH). It was apparently extirpated from Lake Okoboji before 1933, but persisted long after that time in southeastern Iowa (Shimek 1935a, Gale 1975).

DISCUSSION

Iowa historically supported at least 49 species of freshwater gastropods, including 47 taxa recognized as true species and two genera consisting of an undetermined number of species. This diversity compares favorably to taxonomic richnesses in other recently surveyed states that are smaller or similar in size to Iowa, including Connecticut (35 species; Jokinen 1983), Maine (45 species; Martin 1999), New York (61 species; Jokinen 1992), and Virginia (53 species; Stewart and Dillon 2004). Abundant and diverse aquatic habitats enabled relatively high freshwater gastropod diversity in Iowa. An impressive variety of "prosobranchs" (e.g., Viviparidae, Hydrobiidae, and Pleuroceridae) has been recorded from the eastern portion of the Mississippi River watershed. Probable explanations for this rich assemblage include emigrations from the Great Lakes via the Mississippi River and diverse benthic habitats in the abundant rivers of the region (Dazo 1965). Additionally, a very different yet diverse group of "pulmonates" (e.g., Lymnaeidae, Planorbidae) is known from the prairie pothole and kettlehole region of northcentral and northwestern Iowa, where abundant ephemeral and permanent lentic habitats occur (Prior 1991). In contrast, few gastropod taxa have been recorded from southwestern Iowa. Although it appears that little effort has been directed to sampling gastropods of southwestern Iowa, sediments of the loess soils ecoregion that dominate this area readily erode, resulting in streams with heavy silt loads and muddy beds that are inhospitable to most gastropods (Prior 1991).

Lack of attention to freshwater gastropods might also explain the absence or rarity of recent records for some snail taxa. However, surveys from northern Iowa provide strong evidence that gastropod abundance and diversity declined in that region from the beginning to the end of the 20th century. In 1954-1959 and 1979, Bovbjerg and Ulmer (1960) and Bovbjerg *et al.* (1982) found 11 and 12 species of gastropods in Lake Okoboji, respectively. Although diversity in those studies was substantially higher than in 1933-1934, when only one species was recovered, it remained far below the 32 species reported from Lake Okoboji at the beginning of the 20th century (Shimek 1915, 1935a, Bovbjerg *et al.* 1982). Declines in abundance and diversity in the early 20th century were not restricted to Lake Okoboji. Shimek (1935a)

reported declines in the abundance and diversity of freshwater gastropods across the entire kettlehole region of northern Iowa between Spirit Lake, Dickinson County, and Clear Lake, Cerro Gordo County. Severe pollution of rivers and lakes and destruction of wetland habitat were blamed for declines in gastropod abundance and diversity in northern Iowa in the late 19th and early 20th centuries (Shimek 1935a). Because similar habitat degradation and loss occurred in other regions of Iowa, snail abundance and diversity probably declined throughout much of the state during that time period (Shimek 1935a, Euliss and Mushet 1999). However, increased abundance and diversity of gastropods in Lake Okoboji following improvements in water quality in the latter half of the 20th century indicate that many taxa respond favorably and quickly to abatement of pollution (Bovbjerg *et al.* 1982). If modern strategies to protect water quality are successful, the conservation status of many gastropod taxa should improve (Prior *et al.* 2003). Additionally, snails have likely benefited from recent increases in wetland habitat in Iowa, although gastropod responses to wetland creation and restoration have not yet been evaluated (Bishop *et al.* 1998).

Based on rarity or absence of recent records and evidence of local extinctions associated with pollution and habitat loss, I consider 25 of Iowa's freshwater gastropod taxa to be of conservation concern. Of these taxa, *Valvata lewisi*, *Valvata sincera*, *Acella haldemani*, *Stagnicola catascopium*, *Stagnicola emarginata*, *Planorbella truncata*, and *Planorbella umbillicatellus* probably no longer occur in Iowa. These species have localized historic distributions in the state and have not been reported since 1945 or earlier. *Valvata bicarinata*, *Pomatiopsis cincinnatiensis*, *Pomatiopsis lapidaria*, *Bulimnaea megasoma*, *Physa skinneri*, *Gyraulus deflectus*, *Gyraulus circumstriatus*, *Planorbula armigera*, *Ferrissia fragilis*, *Ferrissia parallelus*, and *Laevapex diaphanus* were rarely reported in recent years, and could also be imperiled or extinct in Iowa. Several additional species likely survive today but were eliminated from many former habitats and appear to be less widespread now than formerly, including *Lymnaea stagnalis*, *Stagnicola exilis*, *Stagnicola caperata*, *Aplexa elongata*, *Micromenetus dilatatus*, *Planorbella campanulata*, and *Laevapex fuscus*.

An objective of this review is to facilitate and stimulate additional research that will improve the conservation status of freshwater gastropods in Iowa and North America in general. A comprehensive field survey of Iowa's freshwater gastropods is needed now to determine which species are truly endangered in this state. Comparisons of data from future field surveys with the historic data I summarized here will provide evidence of restricted or shrinking geographic ranges needed to establish legal protection and recovery action plans for imperiled species.

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LITERATURE CITED

Abbott, R. T. 1948. A potential snail host of oriental schistosomiasis in North America (*Pomatiopsis lapidaria*). *Proceedings of the United States National Museum* **98**: 57-68.

Angelo, R. T., M. S. Cringan, and J. E. Fry. 2002. Distributional revisions and new and amended occurrence records for prosobranch snails in Kansas. *Transactions of the Kansas Academy of Science* **105**: 246-257.

Baker, F. C. 1904. New varieties of American lymnaeas. *The Nautilus* **18**: 10-12.

Baker, F. C. 1905. The molluscan fauna of McGregor, Iowa. *The Nautilus* **15**: 249-258.

Baker, F. C. 1911. *The Lymnaeidae of North and Middle America, Recent and Fossil.* Chicago Academy of Sciences Special Publication No. 3, Chicago, Illinois.

Baker, F. C. 1928. *The Fresh Water Mollusca of Wisconsin*, Part I. Gastropoda. Wisconsin Academy of Sciences, Arts, and Letters, Madison, Wisconsin.

Baker, F. C. 1945. *The Molluscan Family Planorbidae.* The University of Illinois Press. Urbana, Illinois.

Bardach, J. E., J. Morrill, and F. Gambony. 1951. Preliminary report on the distribution of bottom organisms in West Lake Okoboji, Iowa. *The Proceedings of the Iowa Academy of Science* **58**: 405-414.

Barnhart, M. C. 1978. Three introduced gastropods in Iowa. *The Nautilus* **92**: 106-107.

Basch, P. F. 1963. A review of the recent freshwater limpet snails of North America (Mollusca: Pulmonata). *Bulletin of the Museum of Comparative Zoology, Harvard University* **129**: 399-461.

Binney, W. G. 1858. *The Complete Writings of Thomas Say.* H. Bailliere, New York.

Binney, W. G. 1865a. Land and fresh water shells of North America, Part III. Ampullaridae, Valvatidae, Viviparidae, fresh water Rissoidae, Cyclophoridae, Truncatellidae, fresh-water Neritidae, Helicinidae. *Smithsonian Miscellaneous Collections* **144**: i-vii, 1-120.

Binney, W. G. 1865b. Land and fresh water shells of North America, Part II. Pulmonata, Limnophila and Thalassophila. *Smithsonian Miscellaneous Collections* **143**: i-ix, 1-161.

Bishop, R. A., J. Joens, and J. Zohrer. 1998. Iowa's wetlands, present and future with a focus on prairie potholes. *Journal of the Iowa Academy of Science* **105**: 89-93.

Bogan, A. E. 1998. Freshwater molluscan conservation in North

America: Problems and practices. *Journal of Conchology Special Publication* **2**: 223-230.

Bovbjerg, R. V. 1965. Feeding and dispersal in the pond snail *Stagnicola reflexa* (Basommatophora: Lymnaeidae). *Malacologia* **2**: 199-207.

Bovbjerg, R. V. 1968. Responses to food in lymnaeid snails. *Physiological Zoology* **41**: 412-423.

Bovbjerg, R. V. and M. J. Ulmer. 1960. An ecological catalog of the Lake Okoboji gastropods. *The Proceedings of the Iowa Academy of Science* **67**: 569-577.

Bovbjerg, R. V., R. L. Dusil, and R. L. Broer. 1982. The gastropods of Lake West Okoboji, Iowa, twenty years later. *The Proceedings of the Iowa Academy of Science* **89**: 62-67.

Bovbjerg, R. V., N. L. Pearsall, and M. L. Brackin. 1970. A preliminary faunal study of the upper Little Sioux River. *The Proceedings of the Iowa Academy of Science* **77**: 177-184.

Brönmark, C. and J. E. Vermaat. 1998. Complex fish-snail-epiphyton interactions and their effects on submerged freshwater macrophytes. *In*: M. Søndergaard and K. Christoffersen, eds., *The Structuring Role of Submerged Macrophytes in Lakes*. Springer-Verlag, New York. Pp. 47-68.

Brown, K. M. 1979a. The adaptive demography of four freshwater pulmonate snails. *Evolution* **33**: 417-432.

Brown, K. M. 1979b. Effects of experimental manipulations on the life history pattern of *Lymnaea stagnalis appressa* Say (Pulmonata: Lymnaeidae). *Hydrobiologia* **65**: 165-176.

Brown, K. M. 1983. Do life history tactics exist at the intraspecific level? Data from freshwater snails. *American Naturalist* **121**: 871-879.

Brown, K. M. 2001. Mollusca: Gastropoda. *In*: J. H. Thorp and A. P. Covich, eds., *Ecology and Classification of North American Freshwater Invertebrates*, Academic Press, New York. Pp. 297-329.

Burch, J. B. 1989. *North American Freshwater Snails*. Malacological Publications, Hamburg, Michigan.

Burch, J. B. and A. S. Van Devender. 1980. Identification of Eastern North American Land Snails: The Prosobranchia, Opisthobranchia and Pulmonata (Actophila). *Transactions of the POETS Society* **1**: 33-80.

Call, R. E. 1880. Fresh-water mollusks. *In*: Iowa Historical Society, ed., *History of Fremont County, Iowa*, Des Moines, Iowa. Pp. 34-36.

Call, R. E. 1882. Note on the geographical distribution of certain mollusks. *American Naturalist* **16**: 400-402.

Call, R. E. 1886. On the genus *Campeloma*, Rafinesque, with a revision of the species, recent and fossil. *Bulletin of the Washburn College Laboratory of Natural History* **1**: 149-165.

Call, R. E. 1894. On the geographic and hypsometric distribution of North American viviparids. *The American Journal of Science* **48**: 132-141.

Carlson, C. A. 1968. Summer bottom fauna of the Mississippi River, above dam 19, Keokuk, Iowa. *Ecology* **49**: 162-169.

Clampitt, P. T. 1960. An ecological reconnaissance of the bottom fauna, Miller's Bay, Lake Okoboji. *The Proceedings of the Iowa Academy of Science* **67**: 553-567.

Clampitt, P. T. 1970. Comparative ecology of the snails *Physa gyrina* and *Physa integra* (Basommatophora: Physidae). *Malacologia* **10**: 113-151.

Clench, W. J. and S. L. H. Fuller. 1965. The genus *Viviparus* (Viviparidae) in North America. *Occasional Papers on Mollusks* **2**: 385-412.

Clench, W. J. and R. D. Turner. 1955. The North American Genus *Lioplax* in the Family Viviparidae. *Occasional Papers on Mollusks* **2**: 1-19.

Coleman, R. W. 1984. A preliminary survey on certain mollusks of Dickinson County, Iowa. *The Proceedings of the Iowa Academy of Science* **91**: 202.

Dazo, B. C. 1965. The morphology and natural history of *Pleurocera acuta* and *Goniobasis livescens* (Gastropoda: Cerithiacea: Pleuroceridae). *Malacologia* **3**: 1-80.

Dillon, R. T., Jr. 2000. *The Ecology of Freshwater Molluscs*. Cambridge University Press, Cambridge, United Kingdom.

Dillon, R. T. and A. R. Wethington. 2004. No-choice mating experiments among six nominal taxa of the subgenus *Physella* (Basommatophora: Physidae). *Heldia* **6**: 1-10.

Dillon, R. T., Jr., A. R. Wethington, J. M. Rhett, and T. P. Smith. 2002. Populations of the freshwater pulmonate *Physa acuta* are not reproductively isolated from American *Physa heterostropha* or *Physa integra*. *Invertebrate Biology* **121**: 226-234.

Dundee, D. S. 1957. Aspects of the biology of *Pomatiopsis lapidaria* (Say) (Mollusca: Gastropoda: Prosobranchia). *Miscellaneous Publications of the Museum of Zoology, University of Michigan* **100**: 1-37.

Eckblad, J. W., N. L. Peterson, and K. Ostlie. 1977. The morphometry, benthos and sedimentation rates of a floodplain lake in pool 9 of the upper Mississippi River. *American Midland Naturalist* **97**: 433-443.

Euliss, N. H., Jr. and D. M. Mushet. 1999. Influence of agriculture on aquatic invertebrate communities of temporary wetlands in the prairie pothole region of North Dakota, USA. *Wetlands* **19**: 578-583.

Euliss, N. H., D. A. Wrubleski, and D. M. Mushet. 1999. Wetlands of the prairie pothole region: Invertebrate species composition, ecology, and management. *In*: D. P. Batzer and S. A. Wissinger, eds., *Invertebrates in Freshwater Wetlands of North America: Ecology and Management*. John Wiley and Sons, Inc., New York. Pp. 471-514.

Freshwater Mollusk Conservation Society. 2005. Committee on the Status and Distribution of Gastropods: The Freshwater Gastropods of North America. Available at: http://www. cofc. edu/~dillonr/fwgnahome. htm 8 February 2005.

Gale, W. F. 1975. Bottom fauna of a segment of pool 19, Mississippi River, near Fort Madison, Iowa 1967-1968. *Iowa State Journal of Research* **49**: 353-372.

Gale, D. D., E. E. Drevs, and M. P. Gross. 1972. The current status of the limnology and bottom fauna of Lakes West and East Okoboji. *The Proceedings of the Iowa Academy of Science* **79**: 17-24.

Haldeman, S. S. 1840-1845. *A Monograph of the Freshwater Univalve Mollusca of the United States: Including Notices of Species in Other Parts of North America*. E. G. Dorsey, Philadelphia.

Hershler, R. 1996. Review of North American aquatic snail genus

Probythinella (Rissoidea: Hydrobiidae). *Invertebrate Biology* **115**: 120-144.

Hershler, R. and F. G. Thompson. 1996. Redescription of *Paludina integra* Say, 1821, type species of genus *Cincinnatia* (Gastropoda: Hydrobiidae). *Journal of Molluscan Studies* **62**: 33-55.

Hershler, R., J. R. Holsinger, and L. Hubricht. 1990. A revision of the North American freshwater snail genus *Fontigens* (Prosobranchia: Hydrobiidae). *Smithsonian Contributions to Zoology* **509**: 1-49.

Hubert, W. H., G. E. Darnell, and D. E. Dolk. 1984. Late-winter abundance and substrate associations of benthos in pool 13, upper Mississippi River. *The Proceedings of the Iowa Academy of Science* **91**: 147-152.

Jokinen, E. H. 1982. *Cipangopaludina chinensis* (Gastropoda: Viviparidae) in North America, review and update. *The Nautilus* **96**: 89-95.

Jokinen, E. H. 1983. *The Freshwater Snails of Connecticut*. State Geological and Natural History Survey of Connecticut, Department of Environmental Protection Bulletin 109, Hartford, Connecticut.

Jokinen, E. H. 1992. *The Freshwater Snails (Mollusca: Gastropoda) of New York State*. New York State Museum Bulletin 482, Albany, New York.

Jude, D. J. 1973. Food and feeding habits of gizzard shad in pool 19, Mississippi River. *Transactions of the American Fisheries Society* **102**: 378-383.

Kater, S. B. and C. R. S. Koneko. 1972. An endogenously bursting neuron in the gastropod mollusc, *Helisoma trivolvis*: Characterization of activity *in vivo*. *Journal of Comparative Physiology* **79**: 1-14.

Kennedy, J. O. and J. G. Miller, III. 1990. A survey of the benthic macroinvertebrates of the Big Spring Basin, Iowa. *The Journal of the Iowa Academy of Science* **97**: 46-54.

Kessel, R. G. and H. W. Beams. 1984. Intracisternal tubules and intramitochondrial filaments in cells of a snail, *Lymnaea stagnalis*. *Tissue and Cell* **16**: 405-410.

Keyes, C. R. 1888. An annotated catalogue of the Mollusca of Iowa. *Bulletin of the Essex Institute* **20**: 61-83.

Martin, S. M. 1999. Freshwater snails (Mollusca: Gastropoda) of Maine. *Northeastern Naturalist* **6**: 39-88.

Meierhoff, M. L. and S. D. Prill. 1982. A survey of the benthic macroinvertebrates of the upper Iowa River basin. *The Proceedings of the Iowa Academy of Science* **89**: 89-98.

Neves, R. J., A. E. Bogan, J. D. Williams, S. A. Ahlstedt, and P. W. Hartfield. 1997. Status of the aquatic mollusks in the southeastern United States: A downward spiral of diversity. *In*: G. W. Benz and D. E. Collins, eds., *Aquatic Fauna in Peril*, Southeast Aquatic Research Institute Special Publication 1, Lenz Design and Communications, Decatur, Georgia. Pp. 43-85.

Pilsbry, H. A. 1886. Notes on some eastern Iowa snails. *American Naturalist* **20**: 72-77.

Pilsbry, H. A. 1898. Notes on new and little-known Amnicolidae. *The Nautilus* **12**: 42-44.

Pratt, W. H. 1876. List of land and fresh water shells found at Davenport, Iowa. *Proceedings of the Davenport Academy of Natural Sciences* **1876**: 165-168.

Prior, J. C. 1991. *Landforms of Iowa*. University of Iowa Press, Iowa City, Iowa.

Prior, J. C., J. L. Boekhoff, M. R. Howes, R. D. Libra, and P. E. VanDorpe. 2003. *Iowa's Groundwater Basics*. Iowa Department of Natural Resources, Iowa City, Iowa.

Rausch, C. G. and R. V. Bovbjerg. 1973. Fauna of the middle Little Sioux River and comparison with upper and lower regions. *The Proceedings of the Iowa Academy of Science* **80**: 111-116.

Say, T. 1817. Descriptions of new species of land and fresh water shells. *Journal of the Academy of Natural Sciences of Philadelphia* **1**: 123-126.

Say, T. 1821. Descriptions of univalve shells of the United States. *Journal of the Academy of Natural Sciences of Philadelphia* **2**: 149-179.

Say, T. 1832. *American Conchology, or Descriptions of the Shells of North America, Illustrated by Colored Figures. Part IV*. New Harmony, Indiana.

Shimek, B. 1890. The Mollusca of eastern Iowa. *Bulletin of the State University of Iowa* **1**: 56-81.

Shimek, B. 1893. Additional notes on Iowa Mollusca. *The Proceedings of the Iowa Academy of Science* **1**: 107-111.

Shimek, B. 1915. The Mollusca of the Okoboji region. *Bulletins from the Laboratories of Natural History* **7**: 70-88.

Shimek, B. 1935a. The effect of pollution on mollusks in Iowa. *The Nautilus* **48**: 109-111.

Shimek, B. 1935b. *Ferrissia* in the lake region of Iowa. *The Nautilus* **49**: 44-46.

Simpson, C. T. 1895. *Pleurocera subulare* in water-mains. *The Nautilus* **9**: 37-38.

Smith, D. G. 2000. Notes on the taxonomy of introduced *Bellamya* (Gastropoda: Viviparidae) species in northeastern North America. *The Nautilus* **114**: 31-37.

Stewart, T. W. and R. T. Dillon, Jr. 2004. Species composition and geographic distribution of Virginia's freshwater gastropod fauna: A review using historical records. *American Malacological Bulletin* **19**: 79-91.

Stimpson, W. 1865. *Researches Upon the Hydrobiinae and Allied Forms*. Smithsonian Miscellaneous Collections 201, Washington, DC.

Te, G. 1975. Michigan Physidae, with systematic notes on *Physella* and *Physodon*. *Malacological Review* **8**: 7-30.

Thompson, D. 1973. Feeding ecology of diving ducks on Keokuk Pool, Mississippi River. *Journal of Wildlife Management* **37**: 367-381.

Thompson, F. G. 1977. The hydrobiid snail genus *Marstonia*. *Bulletin of the Florida State Museum, Biological Sciences* **21**: 113-158.

Thompson, F. G. 1984. North American freshwater snail genera of the hydrobiid subfamily Lithoglyphinae. *Malacologia* **25**: 109-141.

Tobin, G. A. 2000. Geographical overview and configuration: An analysis of the natural landscape. *In*: F. H. Gille, N. Capace, and T. F. Gille, eds., *The Encyclopedia of Iowa*, Vol. 1. Somerset Publishers, Inc., St. Clair Shores, Michigan. Pp. 11-22.

Tryon, G. W. 1863. Notes on American fresh water shells. *Proceed-*

ings of the Academy of Natural Sciences of Philadelphia **1863**: 451-452.

Tryon, G. W. 1865. Catalogue of the Mollusca, collected by Prof. D. S. Sheldon, at Davenport, Iowa. *American Journal of Conchology* **1**: 68-70.

Tryon, G. W. 1870-1871. *A Monograph of the Fresh-water Univalve Mollusca of the United States*. Conchological Section of the Academy of Natural Sciences, Philadelphia, Pennsylvania.

Turgeon, D. D., J. F. Quinn, Jr., A. E. Bogan, E. V. Coan, F. G. Hochberg, W. G. Lyons, P. M. Mikkelsen, R. J. Neves, C. F. E. Roper, G. Rosenberg, B. Roth, A. Scheltema, F. G. Thompson, M. Vecchione, and J. D. Williams. 1998. *Common and Scientific Names of Aquatic Invertebrates from the United States and Canada: Mollusks*. American Fisheries Society Special Publication 26, Bethesda, Maryland.

Ulmer, M. J. 1960. *Physa sayii*, a new intermediate host for the turtle lung fluke, *Heronimus chelydrae* (Trematoda: Heronimidae). *Journal of Parasitology* **46**: 813-814.

Ulmer, M. J. and S. C. Sommer. 1957. Development of sporocysts of the turtle lung fluke, *Heronimus chelydrae* MacCallum (Trematoda: Heronimidae). *The Proceedings of the Iowa Academy of Science* **64**: 601-613.

U. S. Fish and Wildlife Service. 2005. U.S. Listed Invertebrate Animal Species by Taxonomic Group. Available at: http://ecos . fws. gov/tess_public/TESSWebpage 8 February 2005.

Van Cleave, H. J. and R. Chambers. 1935. Studies on the life history of a snail of the genus *Lioplax*. *American Midland Naturalist* **16**: 913-920.

van der Schalie, H. and D. S. Dundee. 1955. The distribution, ecology, and life history of *Pomatiopsis cincinnatiensis* (Lea), an amphibious, operculate snail. *Transactions of the American Microscopical Society* **74**: 119-133.

Walker, B. 1902. A revision of the carinate valvatas of the United States. *The Nautilus* **15**: 121-125.

Walker, B. 1904. Notes on American Ancyli. III. *The Nautilus* **18**: 75-83.

Walker, B. 1909. Notes on *Planorbis* II: *P. bicarinatus*. *The Nautilus* **23**: 21-32.

Walker, B. 1918. A synopsis of the classification of the freshwater Mollusca of North America, North of Mexico, and a catalogue of the more recently described species, with notes. *University of Michigan Museum of Zoology Miscellaneous Publications* **6**: 1-213.

Witter, F. M. 1878. List of Mollusca collected at Muscatine, Iowa. *Journal of Conchology* **1**: 383-394.

Wurtz, C. B. 1949. *Physa heterostropha* (Say). *The Nautilus* **63**: 20-33.

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Experimental studies on habitat preference and tolerances of three species of snails from the Snake River of southern Idaho, U.S.A.

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Abstract: In laboratory experiments we studied the habitat preferences and physical tolerances of two endangered snails, the Utah valvata (*Valvata utahensis*) and the Idaho springsnail (*Pyrgulopsis idahoensis*), and the non-native snail *Potamopyrgus antipodarum*, from the Snake River of southern Idaho, U.S.A., in an attempt to understand habitat use and potential limiting factors in nature. Snails were tested for habitat preference in custom, 1 L aquaria that presented four substrates simultaneously. We tested the snail's tolerance to stream velocity in a laboratory flume capable of delivering water at velocities of approximately 0.15 m/s to 1.0 m/s. We observed tolerance to desiccation and loss of mass in snails by exposing animals to one of three moisture treatments and measuring mass over 50 h. Results show that in laboratory tests individuals of *V. utahensis* prefer pebble substrate types ($\chi^2 = 20.72$, $p < 0.0001$), individuals of *P. idahoensis* use sand substrates most often ($\chi^2 = 2.20$, $p = 0.53$) but preference could not be assigned, and individuals of *P. antipodarum* prefer gravel substrate types ($\chi^2 = 13.58$, $p = 0.004$). Median detachment velocities for snails were significantly different ($\chi^2_{(2)} = 6.19$, $p = 0.045$) being greatest for *P. antipodarum* (0.24 m/s) compared to *V. utahensis* (0.20 m/s) and *P. idahoensis* (0.17 m/s). Tolerance to desiccation differed between treatments of dry, damp, and wet moisture ($F = 80.06$, $p < 0.0001$). Snails lost significant mass in dry treatments after one hour of exposure to desiccating conditions (Dunnett's $p > t < 0.0001$). Very little is known regarding factors controlling the presence of many western North American snail species. Ours is the first experimental study to address habitat use and potential limiting factors controlling the presence of these federally protected snails.

Key words: endangered, habitat preference, invasive species, *Valvata*, *Pyrgulopsis*

Many large river systems, including the Snake River Basin of southern Idaho, have been altered by colonization of the arid American West. There are eight large dam and reservoir projects on the Snake River in Idaho that store water for irrigation and power generation and these have dramatically altered the natural flow regime of the river. From these impoundments approximately 2.2 billion m^3 of water are diverted annually for irrigation. In addition, there are approximately 320 dairies and 80 fish hatcheries between Milner dam and King Hill, Idaho that release effluent into the middle Snake River. Although these effluent releases are regulated by state and federal agencies, the Snake River frequently exceeds total maximum daily limits (TMDLs) for nitrogen, phosphorus, and suspended sediments (USEPA 2002). Further, a recent study of benthic sediments in the middle Snake River revealed high levels of 14 trace elements below irrigation returns and hatchery facilities; eight of which exceeded National Oceanic and Atmospheric Administration's background levels (*i.e.* for salmonid production) (Falter and Hinson 2003). These flow alterations, nutrient additions, and accumulated trace metals in benthic sediments likely threaten the native biota of the Snake River (USFWS 1995). Anthropogenic influences have been shown to alter natural flow regimes; the geomorphology of stream beds; water chemistry; and the quantity, quality, type, and availability of riverine habitats (Cairns *et al.* 1975, Gersich and Brusven 1981, Armitage 1984, Munn and Brusven 1991, Brusven *et al.* 1995, Nelson 1996) and are likely causing similar changes in the Snake River Basin.

Little is known of the life history and ecology of the Utah valvata, *Valvata utahensis* Call, 1884 or the Idaho springsnail, *Pyrgulopsis idahoensis* Pilsbry, 1933, two endangered snails in the Snake River of southern Idaho, USA, which makes managing the Snake River for snail conservation difficult. Further, little is known about how anthropogenic changes to the river affect the survival of these geographically-restricted snails. As a result, management decisions are often based on incomplete information rooted in ecological theory tested with other organisms (Gore 1977, Armitage 1984, Bowler and Frest 1992, Brusven *et al.* 1995). Our work, we aspire, will begin to collect information necessary for sound management decisions and will stimulate additional research interest in the rich malacofauna of the Snake River.

Historically, we believe the Idaho springsnail was distributed in the Snake River from the Oregon border (although fossil evidence only places the westward extent of the species near Homedale, Idaho) to Twin Falls Idaho (Smith 1978, Taylor 1982a, Taylor 1982b). The Utah valvata was historically wide-ranging from Bear Lake in south-eastern

Idaho and north-central Utah through southern Idaho with fossil forms found in parts of central and southern California (Taylor 1985, USFWS 1995). Both species were believed to have experienced an approximately 80% reduction in distributions relative to historic ranges (USFWS 1995). In response to these declines, the United States Fish and Wildlife Service listed *Valvata utahensis* and *Pyrgulopsis idahoensis* as Endangered in 1992 (USFR 1992).

We investigated the substrate preference, current velocity tolerance, and desiccation tolerance in *Valvata utahensis*, *Pyrgulopsis idahoensis*, and one non-native species found in the Snake River, *Potamopyrgus antipodarum* (Gray, 1843). We conducted a substrate preference test to describe preferred substrates for each species and then compared these results to field observations. Substrate is extremely important to benthic organisms (Collier *et al.* 1998, O'Brien and Blinn 1999, Greenwood and Thorp 2001) and a reduction of, or lack of, preferred habitat may have consequences for the survival of the species. An experiment in which we varied current velocity addressed the limits of snails' abilities to stay attached to the substrate and allowed insight into the consequences of rapidly changing stream velocities. A river's current has direct effect on benthic substrates, and rapidly changing or unpredictable stream velocities may have an effect on benthic substrates and the organisms found there (Simons 1979, Poff *et al.* 1997, Watters 1999, Pringle *et al.* 2000). A desiccation tolerance experiment described the snails' abilities to withstand desiccating environmental conditions. Freshwater and marine gastropods are physiologically dependent on submergence in water (*e.g.* for respiration and osmotic balance; Boycott 1936 *in* Dillon 2000) although some species show remarkable tolerance to desiccation (McMahon 1990, Richards *et al.* 2002). Generally, however, stranding above the water-line is detrimental to most aquatic invertebrates (Brusven *et al.* 1974, Brusven 1984, Christman *et al.* 1996). This study provides original baseline information for comparison with field-collected data with the intent of providing information useful to resource managers working on the conservation and recovery of snails in the Snake River.

METHODS

Collection

Snails were collected from known locations in two reservoirs on the main stem of the Snake River in south-central Idaho. Individuals of *Valvata utahensis* were collected in June and August of 2001 and in April and June of 2002 from Lake Walcott Reservoir (42°40′20″N, 113°27′39″W [2001]; 42°40′22″N, 113°25′55″W [2002]). Lake Walcott is part of the Minnidoka National Wildlife Refuge and is managed for fish and wildlife. As a result, the water level does not vary substantially from the 1,278 m surface elevation at full pool. Lake Walcott is approximately 53 km long, covers approximately 4796 ha, and has a mean annual discharge of 240.7 m^3 sec^{-1}. Individuals of *V. utahensis* are generally found between water depths of 2 and 8 m (USGS 2000, USBOR 2003) and in Lake Walcott were collected on fine substrates with mean particle sizes ≤0.5 mm in diameter (USBOR 2003).

Individuals of *Pyrgulopsis idahoensis* were collected in June and July of 2001, and in May and June of 2002 from C. J. Strike Reservoir (42°54′45″N, 115°53′16″W). The reservoir is approximately 56 km long, covers approximately 3076 ha, and has a mean annual discharge of 49.1 m^3 sec^{-1}. C. J. Strike Reservoir is managed primarily for storage of irrigation water and hydro-electric power generation. Therefore, the surface elevation of 748 m at full pool can fluctuate daily and seasonally. For purposes of power generation, C. J. Strike is operated in a load-following capacity whereby water is released during times of peak energy demand, resulting in dramatic stage-level fluctuations immediately below the dam in the main-stem of the Snake River. Collections of *P. idahoensis* in C. J. Strike were from previously known, occupied sites. Substrates at collection sites were primarily coarse sand (0.2-2 mm) to small pebble-sized (1.6-3.2 cm) particles.

We collected snails from random locations within a known colony in both Lake Walcott and C. J. Strike Reservoirs with United States Bureau of Reclamation (USBOR) SCUBA-trained biologists. Snails were suctioned to the lake surface and captured in a 500 μm mesh sieve. Field collections were sorted, placed in polyethylene collection jars, and transported on ice to the laboratory. Snails were kept in four 18 L aquaria in an environmentally-controlled greenhouse facility. Each aquarium was filled with sterilized substrates, de-ionized water spiked with 1 L of Snake River water (to promote the growth of the native aquatic flora), and aerated. Aquaria remained at 19°C (± 2°C) throughout the year and water was run through a carbon-activated filtration system with an ammonium-removing cartridge. The greenhouse was subject to ambient photoperiods and the temperature inside ranged from 15°C to 32°C throughout the year, with relative humidity between 50% and 60%. Voucher specimens of protected species were reposited at the Orma J. Smith Museum of Natural History, Caldwell, Idaho (Accession #s ALBRCIDA 00038038-00038051).

Substrate preference

Aquaria were constructed from clear PVC plastic. Each square aquarium had a bottom that was 225 cm^2 in surface area and was partitioned into four quadrants of 53.3 cm^2 each. The plastic divider separating quadrants had a surface area of 5.8 cm^2 and was 1cm high. Total inside height of each

aquarium was 3 cm (Fig. 1). Substrates were collected from the Boise River (sand, gravel, and pebble) and Lake Walcott Reservoir (silt) and returned to the laboratory for processing. Substrates were dried and sorted by size into silt, sand, gravel, and pebble of increasing and appropriate size according to a modified Wentworth scale (Cummins 1962). Sorted substrates were washed, rinsed, and heated at 100°C for 24 h in a drying oven. We selected three test aquaria randomly from a total of six and introduced substrates randomly to one of the four quadrants. Immediately preceding the start of a replicate test, we mixed silt substrates with de-ionized water to a smooth consistency in a separate glass dish to minimize cross-contamination of substrates between quadrants. Substrates were introduced into test aquaria in order of increasing particle size (silt, sand, gravel, pebble) to an approximate depth of 1 cm. We added de-ionized water to each test aquarium to a depth of 1cm above the substrates (Fig. 1) and then suctioned off suspended particles of silt and sand.

Individual snails of each species were randomly selected from the 18 L general holding tanks, measured for length, and introduced to the intersection of the quadrant dividers (one snail per aquarium). We allowed individuals to sample their environments (each substrate presented) and at 24 h recorded the snail's position as one of the four substrate types (n = 60), giving us a snapshot of substrate use at one point in time. Three tests (one for each species) were run simultaneously and snails were subsequently returned to a different 18 L general holding tank for snails that had been used in experimentation. After completion of a replicate test, substrates were removed, sorted, washed, and dried to be replaced in the supply of similarly-sized substrate material. All substrate preference tests were conducted under a red light. We assumed that (1) the appropriate substrates were presented to snails, while acknowledging that every substrate could not be accommodated by our experimental design and (2) that snails sampled all substrates presented and remained in, or returned to, the preferred one.

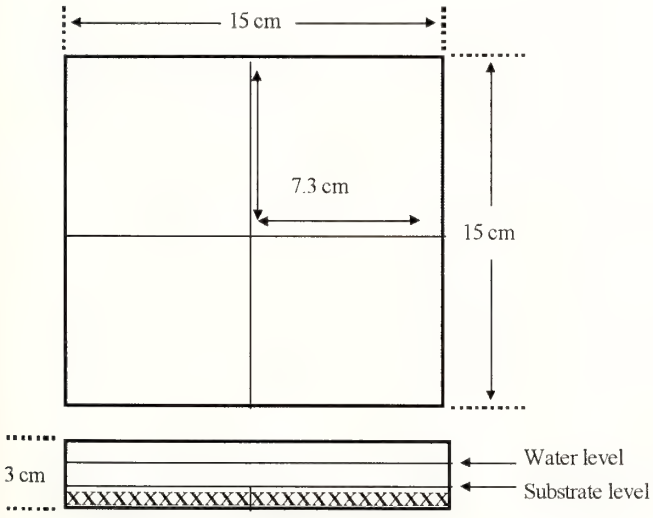

Figure 1. Schematic diagram of aquaria from test for substrate preference. Upper diagram, aquarium floor; bottom diagram, side of aquarium.

The snapshot method of assigning substrate use to an organism's position at a point in time is a commonly used experimental design (Peake 1999, Greenwood and Thorp 2001, van Snik Gray and Stauffer 2001). We also videotaped (Sandpiper Technologies, Sentinel Video Surveillance System, Manteca, California 95337) a sub-set of our replicates for verification of assignment of substrate choice recorded for the 24 h snapshot method. A total of 456 hours of footage were viewed and the average time each snail spent in each substrate was calculated and recorded. We assigned preference to the substrate used most in each 24 h video. For substrate preference we used a chi-square likelihood of occurrence test to compare the observed versus expected occurrence of events. We used contingency analysis to compare substrate use by method (24 h snapshot vs. video). All analyses were conducted with JumpIn (2001) statistical software.

Detachment velocities

We designed an artificial stream reach (flume) and gravity feed reservoir from clear PVC stock material and a 125 L plastic receptacle, respectively (Fig. 2). An open channel flume, square in cross-section (cross-sectional area = 40 cm^2), was approximately 1.5 m long with a 2.5 L weir at the upstream end. A current velocity meter was placed in the channel at the downstream end. Water to the weir was controlled by a 5 cm outside-diameter ball valve. A 5 cm inside-diameter PVC tube connected the 125 L reservoir to the ball valve and the ball valve to the weir. A collimator constructed

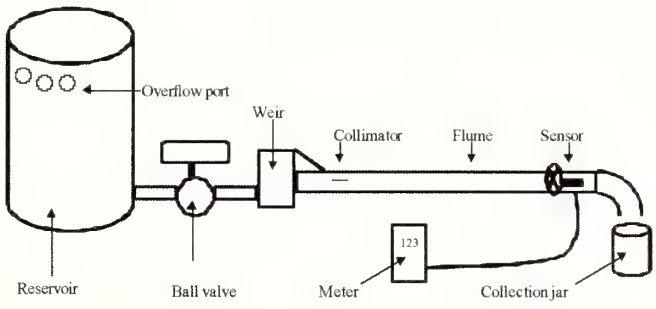

Figure 2. Schematic diagram of reservoir and flume system used for the current velocity test.

of 4 cm-long segments of plastic drinking straws served to decrease the turbulence of water exiting the flume and created a more laminar flow of water inside the flume channel. A down-spout directed water leaving the flume into a 800 ml polypropylene beaker with a 1 mm mesh screen in the bottom to capture detached snails. Brown paper towelettes were attached to the outside of the flume to reduce ambient light intensity. Tap water was supplied to the reservoir. Water levels were held constant in the reservoir (to maintain a constant hydraulic head) via over-flow ports cut into the plastic reservoir. The artificial stream and reservoir delivered water at velocities between 0.14 and 1.0 m sec^{-1} at depths of 4 to 6 cm.

Individual snails were randomly selected from 18 L general holding tanks, measured using a compound microscope with an occular micrometer, and introduced singly to the flume. We allowed snails to attach to the bottom of the flume at a water velocity of 0 m sec^{-1}. Velocities were increased in increments of 0.05 m sec^{-1} every minute until the snail detached from the substrate (n = 25 for *Valvata utahensis* and *Potamopyrgus antipodarum*; n = 33 for *Pyrgulopsis idahoensis*). Current velocity at detachment was the response variable. Current velocity tests were conducted in the greenhouse facility at ambient conditions. We made several assumptions regarding experimental design including: (1) municipal tap-water does not affect detachment response, (2) detachment was the result of water velocity directly and not some combination of velocity and fatigue, and (3) the volume of water in the flume did not affect detachment response.

Because data were not normally distributed (Ramsey and Schafer 1997) and data transformations failed to normalize the values, we used a Kruskal-Wallis test for *k* independent samples (SAS 2001) to compare median detachment velocities between species.

Desiccation tolerance

We addressed the potential effects of desiccating conditions on the survivorship of snails. We exposed snails to one of three treatments that mimicked potential conditions experienced during reservoir draw-down. Each experimental unit consisted of a 47 mm-diameter petri dish lined with filter paper and a single snail. A wet treatment was filled to 1 cm with de-ionized (DI) water, a damp treatment had the filter paper saturated with DI, and a dry treatment was left completely dry.

Thirty individuals each of *Valvata utahensis* and *Pyrgulopsis idahoensis* were chosen for each treatment from the 18 L general holding tanks, blotted dry, weighed to the nearest 0.0001 g, and randomly assigned to one of three treatments (n = 10). Snails were removed from the petri dishes every hour for 10 successive hours, blotted dry (if in wet or damp treatments), and weighed. After 10 h, snails were removed, blotted, and weighed at hours 20, 30, 40, and 50, after which time the experiment concluded. Given the endangered status of the animals, we attempted to limit mortality by removing the snails from the wet treatment after 20 h and placing them in a recovery chamber. For this design we assumed that submerged snails (our control) lost no mass between 20 and 50 h and would not become desiccated, they might have suffered from nutritional deprivation, however, so to prevent additional mortality, snails in the wet treatment were removed early. After 50 h all snails from remaining treatments were removed from petri dishes and placed into recovery chambers with de-ionized water for up to 12 hours. Recovery chambers consisted of 1 L glass containers with aerated, fresh water and processed algae for food. Those snails not active after 12 h in the recovery chamber were considered dead and were preserved in 70% ethanol. We recorded mass loss and calculated percentage of mass loss.

In the desiccation tolerance test we used a 3 × 2 × 12 mixed factorial design. We used the GLM procedure in SAS (2001) to examine main effects of species, treatment, and time, as well as interactions between factors. We employed the Dunnett's test for means comparisons with time "one" (initial mass) as the control. We used transformed data for this test because we needed to compare all factors simultaneously but were unable to standardize snail mass at the beginning of the experiment. We used a Students t-test on percent data to test the null hypothesis of no difference in total mass lost between *Valvata utahensis* and *Pyrgulopsis idahoensis* in the dry treatment only. Assumptions of the t-test were met and therefore data were not transformed. All analyses were conducted with SAS statistical software (SAS 2001).

RESULTS

Substrate preference

Two observations for *Pyrgulopsis idahoensis* were removed. One snail did not move from the intersection of the substrate dividers for the entire 24 h period and one snail died during the 24 h test. One observation for *Valvata utahensis* was removed because the snail was found clinging to the surface of the water at the 24 h mark. Thus, it could not be assigned to any substrate type. Mean sizes of snails used in the substrate preference test was 4.6 mm (3.0-5.6 mm) for *V. utahensis*, 5.6 mm (4.0-7.0 mm) for *P. idahoensis*, and 5.1 mm (3.9-5.7 mm) for *Pyrgulopsis antipodarum*.

We found significant differences between observed and expected values for substrate use by *Valvata utahensis* and *Potamopyrgus antipodarum*, but not by *Pyrgulopsis idahoensis* (Table 1, Fig. 3). Individuals of *V. utahensis* selected the pebble habitat type in 31 of 59 replicates and showed pref-

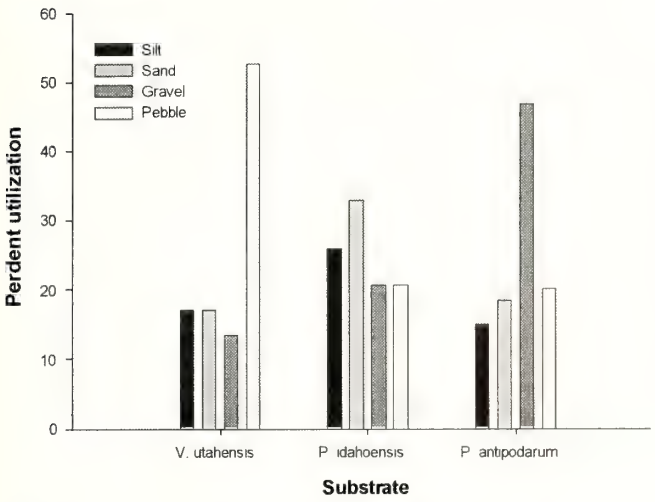

Figure 3. Percent utilization by *Valvata utahensis* (n = 59), *Pyrgulopsis idahoensis* (n = 58), and *Potamopyrgus antipodarum* (n = 60) for each substrate type. * Differences are significant ($p < 0.05$), pair-wise comparisons were not conducted.

erence for the largest substrate presented in our study ($p < 0.01$). Individuals of *P. antipodarum* selected the gravel substrate type in 28 of 60 replicates, significantly more than any other substrate ($p < 0.01$), and individuals of *P. idahoensis* utilized all substrates similarly ($p = 0.52$). We found that our designation of substrate preference was independent of the method used (24 h snapshot vs. video), which supported our experimental results from the snapshot method ($\chi^2 = 3.29$, $p = 0.35$). Caution is suggested, however, as designation of substrate preference was the same in only 53% of replicates that were both videotaped and assigned preference by the snapshot method (n = 19). Although we accept the null hypothesis of no difference, the low percentage of agreement may be a function of sample size or may be because contingency analyses are conservative in providing protection against Type I error.

Detachment velocities

Mean sizes of snails used in the current velocity test was 4.6 mm (3.2-5.6 mm) for *Valvata utahensis*, 5.5 mm (4.0 mm-6.9 mm) for *Pyrgulopsis idahoensis*, and 5.1 mm (4.0 mm to 5.6 mm) for *Potamopyrgus antipodarum*. Median detachment velocities for *V. utahensis*, *P. idahoensis*, and *P. antipodarum* were significantly different ($\chi^2_{(2)} = 6.19$, $p = 0.045$). Median detachment velocities were 0.20 m s^{-1} (n = 33) for *V. utahensis*, 0.17 m s^{-1} (n = 31) for *P. idahoensis*, and 0.24 m s^{-1} (n = 25) for *P. antipodarum* (Figure 4). Maximum detachment velocities were 0.41 m s^{-1} for *V. utahensis*, 0.58 m s^{-1} for *P. idahoensis*, and 0.51 m s^{-1} for *P. antipodarum*.

Table 1. Source table for χ^2 likelihood of occurrence analysis from substrate preference tests. Results indicated that observed differences in substrate use by *Valvata utahensis* and *Potamopyrgus antipodarum* were not likely due to chance but those of *Pyrgulopsis idahoensis* may have been.

	Silt	Sand	Gravel	Pebble	χ^2	Pr > χ^2
V. utahensis						
Observed	10	10	8	31	20.72	0.0001
Expected	14.75	14.75	14.75	14.75		
P. idahoensis						
Observed	15	19	12	12	2.20	0.53
Expected	14.5	14.5	14.5	14.5		
P. antipodarum						
Observed	9	11	28	12	13.58	0.004
Expected	15	15	15	15		

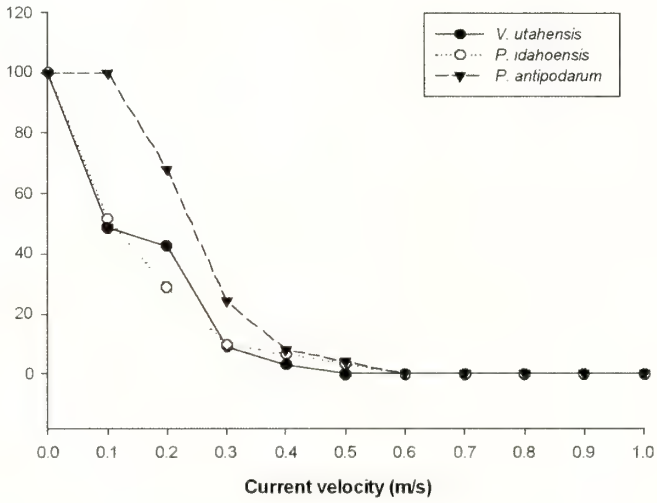

Figure 4. Percent of snails remaining at discrete water velocity intervals between 0 and 1.0 m sec^{-1} (*Valvata utahensis* n = 31, *Pyrgulopsis idahoensis* n = 33, *Potamopyrgus antipodarum* n = 25).

Desiccation tolerance

The main effect of treatment on mass loss was significant ($F_{(2, 54)} = 80.6$, $p < 0.0001$), as was the difference in mass over time ($F_{(11, 594)} = 9.75$, $p = < 0.0001$). The interaction between treatment and time was also significant ($F_{(22, 594)} = 11.06$, $p < 0.0001$). There were no significant differences in the mass lost between species, species by time, or treatment by species by time (Table 2). From Dunnett's test we found that individuals of both *Valvata utahensis* and *Pyrgulopsis idahoensis* lost significant mass after only 1h ($p < 0.0001$) in the dry treatment. Data from hours 30, 40, and 50 in the damp and wet treatments were not compared statistically. The mean percent of mass lost after 20 h for *V. utahensis* was 1.70 (± 4.0) in the wet treatment, 1.92 (± 3.07)

Table 2. Sources of variation from repeated measures ANOVA on mass loss in the desiccation experiment.

Source	DF	SS	MS	F	P
Treatment	2	17356	8677.77	80.06	<0.0001
Species	1	144.27	144.27	1.33	0.25
Treatment × species	2	154.50	77.25	0.71	0.49
Error	54	5852.97	108.39		
Snail (treatment × species)	54	5852.97	108.39	6.95	<0.0001
Time	11	1672.77	152.07	9.75	<0.0001
Time × treatment	22	3795.99	172.55	11.06	<0.0001
Time × species	11	136.48	12.41	0.80	0.64
Time × treatment × species	22	437.13	19.87	1.27	0.18
Error (time)	594	9266.35	15.60		

in the damp treatment, and 11.59 (± 3.75) in the dry treatment. For *P. idahoensis* the mean percent of mass change after 20 h was a positive 2.99 (± 1.36) in the wet treatment. The mean percent mass lost in *P. idahoensis* after 20 h was 0.23 (± 1.01) in the damp treatment and 19.91 (± 7.02) in the dry treatment. We found that individuals of *P. idahoensis* lost significantly more mass in the dry treatment compared to individuals of *V. utahensis* ($t_{(18)} = 3.30$, $p = 0.004$).

DISCUSSION

Habitat choice by snails is likely a function of several environmental variables including the quality of food resources (Eisenberg 1970, Lodge 1986), particle size of the substrate (Clampitt 1973, Harman 1974), current velocity (Johnson and Brown 1997, Holomuzki and Biggs 1999), inter- or intraspecific competition, or the perceived risk of predation (Hershey 1990). When given a choice, different species of snails in our study selected different substrates. Individuals of *Valvata utahensis* and *Potamopyrgus antipodarum* preferred pebble and gravel substrates, respectively, more than other substrates presented. Individuals of *Pyrgulopsis idahoensis* utilized sand substrates more than silt, gravel, or pebble but the differences were not significant and preference is not assigned. Interestingly, all three species may be found sympatrically in the Snake River and show partitioning of substrate use in a laboratory setting that affords the question of whether there is niche overlap in nature. Field data suggest that there is niche overlap, but only by native and non-native snails (IPC 2003, USBOR 2003).

Individuals of *Valvata utahensis* have generally been observed in deep water habitats (2-8 m) with silt substrates (Taylor 1982a, Frest and Johannes 1992, USBOR 2002). However, they have also been found in shallow waters (<2 m) in free-flowing reaches of the Snake River on larger-diameter substrates (USBOR 2003) similar to substrates they appear to prefer in this study. By contrast, individuals of *Pyrgulopsis idahoensis* are generally found in less than 2 m of water on sand to gravel-sized substrates in reservoirs, similar to the results of this study, or on gravel to cobble-sized substrates in the main-stem Snake River (IPC 2004). Individuals of *V. utahensis* and *P. idahoensis* may be sympatric in the Snake River but generally use slightly different habitats (*e.g.*, substrate and depth; USBOR 2003, 2004). Individuals of *Potamopyrgus antipodarum* appear to utilize all available habitat types (S. Lysne, pers. obs.) and reach great densities (Richards *et al.* 2001, Hall *et al.* 2003). In this study, snails were presented with substrate types found in nature but were not presented with cobble-sized substrates or vegetation. Cobble is too large and vegetation would have introduced greater vertical structure than the experimental design could accommodate. Thus it is possible that we did not present important substrates and this may partially explain why there was no clearly preferred substrate in the laboratory for individuals of *P. idahoensis*. The genus *Pyrgulopsis* is extremely diverse, particularly in the western United States (Burch 1989, Hershler 1994, Ponder and Colgan 2002), and it is reasonable that species have evolved highly specific habitat preferences. For example, O'Brien and Blinn (1999) studied habitat utilization by *Pyrgulopsis montezumensis* Hershler, 1988 and found it to prefer limestone substrates with extremely high CO_2 concentrations. Mladenka (1992) studied the habitat requirements of *Pyrgulopsis bruneauensis* Hershler, 1990, a small hydrobiid that exists only in thermally influenced sites with water temperatures between 20°C and 30°C. These observations of highly specific habitat preferences of congeners and our results for *P. idahoensis* suggest that the preferred substrate in nature may not have been presented in this laboratory study.

Compared to native snails, individuals of *Potamopyrgus antipodarum* appear to use a wider range of substrate types. They have been collected in main-stem river and reservoir sites on various substrates (USBOR 2003), from irrigation ditches (Cada 2001), cold-water springs (Richards *et al.* 2001), and comparatively warmer rivers in Yellowstone National Park (Hall *et al.* 2003). These collections suggest that North American *P. antipodarum* are relative habitat generalists even though a significant preference for gravel substrate types was found in our study. Factors other than substrate influence habitat choice (*e.g.*, current velocity, dissolved oxygen, or food availability) (Dillon 2000) and our observations may represent the difference between a fundamental and realized niche.

In nature, current velocity may also play a role in habitat choice by snails. High velocities can dislodge substrates

and cause direct mortality to individuals or reduce population sizes (Scarsbrook and Townsend 1993). We found a significant difference in median detachment velocities between species tested. *V. utahensis* and *Pyrgulopsis idahoensis* had similar median detachment velocities (0.20 m s^{-1}, 0.17 m s^{-1}, respectively) while that of *Potamopyrgus antipodarum* was slightly greater (0.24 m s^{-1}). Holomuzki and Biggs (2000), also working with *P. antipodarum*, showed similar detachment velocities of 0.2 m s^{-1} for greater than 50% of experimental animals. These results, although statistically significant, may not be biologically significant and may represent the physical tolerance of freshwater snails of this size in general.

Studies have demonstrated the effect of hydrologic alterations on benthic invertebrates (Brusven *et al.* 1974, Pringle *et al.* 2000), but to assign cause is difficult. Christmen *et al.* (1996) studied stranding due to fluctuations in stage level in the Coosa River, Alabama. They marked snails approximately one month before the stage level of the river dropped and came back shortly afterward to re-observe marked animals. They found that 93% of recovered individuals of *Tulotoma magnifica* Conrad, 1834 that had been stranded died. Only 7% of recovered snails had migrated to the new water's edge and survived. Our laboratory studies showed that desiccating conditions significantly affected snails. We found that at least half of the snails of both species in the "dry treatment" died within 50 h. We did, however, also find differences in the wet and damp treatments for *Valvata utahensis* and *Pyrgulopsis idahoensis*, but contend these treatment differences are an artifact of methodology and not biologically significant. There was no mortality in either the wet or damp treatment for either species in our study; however, mortality in the dry treatment was 50% for *V. utahensis* and 80% for *P. idahoensis* at 50 h. This is significant because if snails can exploit wet or damp microhabitats during daily drops in stage level then significant mortality to the species may be avoided.

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LITERATURE CITED

Armitage, P. D. 1984. Environmental changes induced by stream regulation and their effect on lotic macroinvertebrate communities. *In*: A. Lillehammer and S. J. Saltveit, eds., *Regulated Rivers*. Engers Boktrykkeri Als, Norway. Pp. 139-162.

Bowler, P. A. and T. J. Frest. 1992. The non-native snail fauna of the middle Snake River, southern Idaho. *Proceedings of the Desert Fisheries Council* **23**: 28-44.

Brusven, M. A. 1974. Benthic insects: Effects of water fluctuations on benthic insects. *In*: *Anatomy of a River*. Pacific Northwest Basin River Commission, Vancouver, Washington. Pp. 67-79.

Brusven, M. A. 1984. The distribution and abundance of benthic insects subjected to reservoir-release flows in the Clearwater River, Idaho, USA. *In*: A. Lillehammer and S. J. Saltveit, eds., *Regulated Rivers*. Engers Boktrykkeri Als, Norway. Pp. 167-180.

Brusven, M. A., D. J. Walker, K. M. Painter, and R. C. Biggam. 1995. Ecological-economic assessment of a sediment-producing stream behind Lower Granite Dam on the lower Snake River, USA. *Regulated Rivers: Research and Management* **10**: 373-387.

Burch, J. B. 1989. *North American Freshwater Snails*. Malacological Publications, Hamburg, Michigan.

Cada, C. 2001. Effects of New Zealand mudsnails on native invertebrates in Darlington Ditch, Montana. *In*: Minutes of the First Annual Conference on New Zealand Mudsnails in the Western USA. 27-28 August. Bozeman, Montana. Chavez Writing and Editing, Inc., Boise, ID. Pp. 4-5.

Call, R. E. 1884. On the quaternary and recent mollusca of the Great Basin, with descriptions of new forms. *U.S. Geological Survey Bulletin* **11**: 1-64.

Cairns, J. Jr., A. G. Heath, and B. C. Parker. 1975. The effects of temperature upon the toxicity of chemicals to aquatic organisms. *Hydrobiologia* **47**: 135-171.

Christman, S. P., E. L. Mihalcik, and F. G. Thompson. 1996. *Tulotoma magnifica* (Conrad 1834) (Gastropoda: Viviparidae) population status and biology in the Coosa River, Alabama. *Malacological Review* **29**: 17-63.

Clampitt, P. T. 1973. Substratum as a factor in the distribution of pulmonate snails in Douglas Lake, Michigan. *Malacologia* **12**: 379-399.

Collier, K. J., R. J. Wilcock, and A. S. Meredith. 1998. Influence of substrate type and physico-chemico conditions on macroinvertebrate faunas and biotic indices of some lowland Waikato, New Zealand, streams. *New Zealand Journal of Marine and Freshwater Research* **32**: 1-19.

Cummins, K. W. 1962. An evaluation of some techniques for the collection and analysis of benthic samples with special emphasis on lotic waters. *American Midland Naturalist* **67**: 477-504.

Dillon, R. T. 2000. *The Ecology of Freshwater Molluscs*. Cambridge University Press, Cambridge, U.K.

Eisenberg, R. M. 1970. The role of food in the regulation of the pond snail, *Lymnaea elodes*. *Ecology* **51**: 680-684.

Falter, C. M. and D. R. Hinson. 2003. *Sediment and Benthic Community Characterization below Agriculture and Aquaculture Waste Loadings in the Middle Snake River, South-Central Idaho.* Unpublished Report for the U.S. Environmental Protection Agency and U.S. Fish and Wildlife Service.

Frest, T. J. and E. J. Johannes. 1992. *Distribution and Ecology of the Endemic Relict Mollusc Fauna of Idaho TNC's Thousand Springs Preserve*. Unpublished report to Idaho Nature Conservancy, SunValley, Idaho.

Gersich, F. M. and M. A. Brusven. 1981. Insect colonization rates in near-shore regions subjected to hydroelectric power peaking flows. *Journal of Freshwater Ecology* **1**: 231-236.

Gore, J. A. 1977. Reservoir manipulations and benthic macroinvertebrates in a prairie river. *Hydrobiologia* **55**: 113-123.

Greenwood, K. S. and J. H. Thorp. 2001. Aspects of ecology and conservation of sympatric, prosobranch snails in a large river. *Hydrobiologia* **455**: 229-236.

Hall, R. O. Jr., J. L. Tank, and M. F. Dybdahl. 2003. Exotic snail dominate nitrogen and carbon cycling in a highly productive stream. *The Ecological Society of America* **1**: 407-411.

Harman, W. N. 1974. Benthic substrates: Their effect on freshwater Mollusca. *Ecology* **53**: 271-277.

Hershey, A. E. 1990. Snail populations in arctic lakes: Competition mediated by predation? *Oecologia* **82**: 26-32.

Hershler, R. 1994. A review of the North American freshwater snail genus *Pyrgulopsis* (Hydrobiidae). *Smithsonian Contributions to Zoology* **554**: 1-115.

Holomuzki, J. R. and B. J. F. Biggs. 1999. Distributional responses to flow disturbance by a stream-dwelling snail. *Oikos* **87**: 36-47.

Holomuzki, J. R. and B. J. F. Biggs. 2000. Taxon-specific responses to high-flow disturbance in streams: implications for population persistence. *Journal of the North American Benthological Society* **19**: 670-679.

Idaho Power Company [IPC]. 2003. *Responses to FERC Additional Information Request 9: Listed Snails-Cove Creek*. Unpublished report, Idaho Power Company, Boise, Idaho. 38 pp.

Idaho Power Company [IPC]. 2004. *Snake River Aquatic Macroinvertebrate and ESA Snail Survey*. Unpublished report, Idaho Power Company, Boise, Idaho. 62 pp.

Johnson, P. D. and K. M. Brown. 1997. The role of current and light in explaining the habitat distribution of the lotic snail *Elimia semicarinata* (Say). *Journal of the North American Benthological Society* **16**: 545-561.

JumpIn V. 4.04. 2001. SAS Institute Inc. Cary, North Carolina.

Lodge, D. M. 1986. Selective grazing on periphyton: A determinant of freshwater gastropod microdistributions. *Freshwater Biology* **16**: 831-841.

McMahon, R. F. 1990. Thermal tolerance, evaporative water loss, air-water oxygen consumption and zonation of intertidal prosobranchs: A new synthesis. *Hydrobiologia* **193**: 241-260.

Mladenka, G. C. 1992. *The Ecological Life History of the Bruneau Hotsprings Snail* (Pyrgulopsis bruneauensis). M.S. Dissertation, Idaho State University, Pocatello, Idaho. 115 pp.

Munn, M. D. and M. A. Brusven. 1991. Benthic macroinvertebrate communities in nonregulated and regulated waters of the Clearwater River, Idaho, U.S.A. *Regulated Rivers* **6**: 1-11.

Nelson, J. M. 1996. Predictive techniques for river channel evolution and maintenance. *Water, Air and Soil Pollution* **90**: 321-333.

O'Brien, C. and D. W. Blinn. 1999. The endemic spring snail *Pyrgulopsis montezumensis* in a high CO_2 environment: Importance of extreme chemical habitats as refugia. *Freshwater Biology* **42**: 225-234.

Peake, S. 1999. Substrate preferences of juvenile hatchery-reared lake sturgeon, *Acipenser fulvescens*. *Environmental Biology of Fishes* **56**: 367-374.

Pilsbry, H. A. 1933. Aminicolidae for Wyoming and Oregon. *Nautilus* **47**: 9-12.

Poff, N. L., N. J. Voelz, and J. V. Ward. 1990. Algal colonization under four exprimentally-controlled current regimes in a high mountain stream. *Journal of the North American Benthological Society* **9**: 303-318.

Poff, N. L., J. D. Allan, M. B. Bain, J. R. Karr, K. L. Prestegaard, B. D. Richter, R. E. Sparks, and J. C. Stromberg. 1997. The natural flow regime a paradigm for river conservation and restoration. *BioScience* **47**: 769-782.

Ponder, W. F. and D. J. Colgan. 2002. What makes a narrow-range taxon? Insights from Australian freshwater snails. *Invertebrate Systematics* **16**: 571-582.

Pringle, C. M., M. C. Freeman, and B. J. Freeman. 2000. Regional effects of hydrologic alterations on riverine macrobiota in the New World: tropical-temperate comparisons. *BioScience* **50**: 807-823.

Ramsey, F. L. and D. W. Schafer. 1997. *The Statistical Sleuth*. Wadsworth Publishing Company, Belmont, California.

Richards, D. C., L. D. Cazier, and G. T. Lester. 2001. Spatial distribution of three snail species, including the invader *Potamopyrgus antipodarum*, in a freshwater spring. *Western North American Naturalist* **61**: 375-380.

Richard, D. C., P. O'Connell, W. P. Dwyer, B. L. Kerans, and D. Cazier Shinn. 2002. *Desiccation and Freezing Mortality Rates of New Zealand Mudsnail*, Potamopyrgus antipodarum. Unpublished report by: EcoAnalysts Research Laboratory, Bozeman Montana.

SAS. 2001. Version 8.0. SAS Institute Inc. Cary, North Carolina.

Scarsbrook, M. R. and C. R. Townsend. 1993. Stream community structure in relation to spatial and temporal variation: A habitat template study of two contrasting New Zealand streams. *Freshwater Biology* **29**: 395-410.

Simons, D. B. 1979. Effects of stream regulation on channel morphology. *In*: J. V. Ward and J. A. Stanford, eds., *The Ecology of Regulated Streams*, Plenum Press, New York. Pp. 95-111.

Taylor, D. W. 1982a. *Status Report on Homedale Creek Spring Snail*. Unpublished report submitted to U.S. Fish and Wildlife Service, Boise, Idaho.

Taylor, D. W. 1982b. *Status Report on the Utah Valvata Snail in Southwestern Idaho*. Unpublished report submitted to U.S. Fish and Wildlife Service, Boise, Idaho.

Taylor, D. W. 1985. Evolution of freshwater drainages and molluscs in Western North America. *In*: Pacific Division, American Association for the Advancement of Science. Pp. 265-321.

Tomanek, L. and G.M. Somero. 1999. Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (genus *Tegula*) from different thermal habitats: Implications for limits of thermotolerance and biogeography. *Journal of Experimental Biology* **202**: 2925-2936.

U.S. Bureau of Reclamation (USBOR). 2002. *Research, Monitoring and Surveys for Snails Protected under the Endangered Species Act in the Upper Snake River, Idaho.* Unpublished report to U.S. Fish and Wildlife Service, Boise, Idaho.

U.S. Bureau of Reclamation (USBOR). 2003. *Monitoring and Distribution Surveys for Utah Valvata Snails Protected under the Endangered Species Act in the Upper Snake River, Idaho.* Unpublished report to U.S. Fish and Wildlife Service, Boise, Idaho.

U.S. Environmental Protection Agency (USEPA). 2002. *Final Environmental Impact Statement for Hydropower license.* Project No. 2055; Federal Energy Regulatory Commission, Office of Environmental and Engineering Review, Washington, D.C.

U.S. Fish and Wildlife Service (USFWS). 1995. *Snake River Aquatic Species Recovery Plan.* Snake River Basin Office, Ecological Services, Boise, Idaho.

U.S. Federal Register (USFR). 1992. Endangered and threatened wildlife and plants: Determinations of endangered or threatened status for five aquatic snails in south central Idaho. Federal Register **57**: 59244-59256.

U.S. Geological Survey (USGS). 2000. *Population Monitoring for* Valvata utahensis *in Lake Walcott, Idaho.* Unpublished report submitted to U.S. Fish and Wildlife Service, Boise, Idaho.

van Snik Gray, E. and J. R. Stauffer Jr. 2001. Substrate choice by three species of darters (Teleostei: Percidae) in an artificial stream: Effects of a nonnative species. *Copeia* **1**: 254-261.

Watters, G. T. 2000. Freshwater mussels and water quality: a review of the effects of hydrologic and instream habitat alterations. *Proceedings of the First Mollusk Conservation Society Symposium* **1999**: 1-13.

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Effects of extracts of the bark of the stem of *Croton tiglium* on the metabolism of the freshwater gastropod *Lymnaea acuminata*

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Abstract: Aqueous extracts of the bark of the stem of the plant *Croton tiglium* (Euphorbiaceae) was found to have strong molluscicidal activity against the snail *Lymnaea acuminata*. Exposure of low doses of aqueous extracts of the bark for 96 h significantly altered the levels of total protein, total free amino acid, glycogen, nucleic acids, pyruvate, lactate, and the activity of the enzymes acetylcholinesterase, lactic dehydrogenase, cytochrome oxidase, protease, succinic dehydrogenase, and acid and alkaline phosphatase in nervous tissue, the hepatopancreas, and the ovotestis of the freshwater snail *Lymnaea acuminata*. The effect in all the cases was dose-dependent. A second study showed that there was significant recovery in the snail's tissues after the seventh day of withdrawal of treatment.

Key words: Enzyme activity, Euphorbiaceae, metabolism

The latex and bark of the stem of the plant *Croton tiglium* (Linnaeus, 1764) (family Euphorbiaceae) have potent molluscicidal activity against the freshwater snails *Lymnaea* (*Radix*) *acuminata* (Lamarck, 1822) and *Indoplanorbis exustus* (Deshayes, 1834) (Yadav and Singh 2001). Both species of snails are the intermediate hosts of the liver flukes *Fasciola hepatica* (Linnaeus, 1788) and *Fasciola gigantica* (Cobbold, 1884), which cause endemic fascioliasis in the cattle and livestock of the northern part of India (Singh and Agarwal 1981).

Croton tiglium is a well-known therapeutic plant of India. The bark of this plant is used in tonic and the seeds are the strongest known purgative and a source of croton oil. Fourteen different phorbol-diesters are known from *C. tiglium*. The use of phorbol esters as a tool in pharmacological studies represents an example of a valuable application of a toxic substance from a species employed for a very different purpose in primitive societies, one of the hallmarks of ethnopharmacological research (Uphof 1968).

The active moiety of extracts present in the environment enters animals through their external surfaces, digestive tracts, circulatory system, or respiratory tracts. They are then carried to different organs of the body by the circulatory system and they may or may not be metabolized depending upon their chemical nature. These toxic materials and their active metabolic products may be retained in various tissues and have a wide variety of sub-lethal effects.

We are interested in the mode of action and long-term effects of the plant products on the target snail *Lymnaea acuminata* and their potential commercial application. The present study reports the effects of low doses of stem bark extracts of the bark of the stem of *Croton tiglium* on the levels of total protein, total free amino acid, glycogen, nucleic acids, pyruvate, and lactate and on the activity of the enzymes acetylcholinesterase, lactic dehydrogenase, cytochrome oxidase, protease, succinic dehydrogenase, and acid and alkaline phosphatase on the brain (nervous tissue), hepatopancreas (digestive gland tissue), and ovotestis (reproductive tissue) of the freshwater snail *Lymnaea acuminata*.

MATERIALS AND METHODS

The bark of the stem of the plant *Croton tiglium* (Linnaeus, 1764) (family Euphorbiaceae) was collected from the Botanical Garden of Deen Dayal Uppadhaya Gorakhpur University, Gorakhpur, India.

Preparation of aqueous extracts of bark: Bark of the stem was dried at 37°C in an incubator and pulverized in a mortar and pestle. The dried powder (10 mg/ml) was homogenized with distilled water for 5 min and centrifuged at 1000 g for 10 min. The supernatant was used as an extract for the following studies.

Experimental conditions of the water were determined using the methods of APHA/AWWA/WPCF (1980). Atmospheric and water temperatures ranged from 30.5-31.5°C and 27.0-28°C, respectively, pH of the water was 7.3-7.5, and dissolved oxygen, free carbon dioxide, and bicarbonate alkalinity ranged from 6.8-7.6, 4.4-6.5, and 105.0-109.0 mg/l, respectively.

Adult individuals of *Lymnaea acuminata* (2.6 ± 0.3 cm in shell height) were collected from Ramgarh Lake of Gorakhpur District, India, and maintained in a plastic tank for acclimatization to laboratory conditions. The acclimatized animals were treated with the extract of *Croton tiglium* according to Singh and Agarwal (1988). The LC_{50} of aqueous

extracts of *C. tiglium* for the snail *L. acuminata* was 6.11 mg dry weight per liter (dw/l) for 96 h of exposure (Yadav and Singh 2001). Individuals of *L. acuminta* were treated with sub-lethal doses of 40% and 80% of the 96 h LC_{50} (2.44 mg and 4.88 mg) of bark extract for 96 h. Six aquaria were set up for each dose and each aquarium contained 30 snails in 6 L dechlorinated tap water.

After completion of the treatment, the test animals were removed from the aquaria and washed with water. The brain, hepatopancreas, and ovotestis were excised and used for biochemical analysis. Control animals were held in similar conditions without any treatment.

To observe the effect of withdrawal from treatment, some snails were first treated with 4.88 mg for exposure periods of 96 h and then were transferred into extract-free water. This water was changed every 24 h for the next 7 days, after which biochemical parameters were measured for the different tissues. Each experiment was replicated at least six times and the values have been expressed as mean ± SE of six replicates. Student's t test and analysis of variance (Sokal and Rohlf 1973) were used to identify significant changes.

BIOCHEMICAL ANALYSES

Protein

Protein levels were estimated according to the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. Homogenates (5 mg/ml w/v) were prepared in 10% TCA.

Total free amino acids

Estimation of total free amino acids was made according to the method of Spices (1957). Homogenates (10 mg/ml w/v) were prepared in 95% ethanol, centrifuged at 6000 g, and used for estimating the content of amino acids.

Nucleic acids

Estimation of DNA and RNA were performed using the methods of Schneider (1957) using diphenylamine and orcinol reagents, respectively. Homogenates (1 mg/ml, w/v) were prepared in 5% TCA at 90°C and centrifuged at 5000 g for 20 min. The supernatant was used for estimation.

Glycogen

Glycogen was estimated by the anthrone method of Van Der Vies (1954) as modified by Mahendru and Agarwal (1982) for snails. In the present experiment, 50 mg of tissue were homogenized with 5 ml of cold 5% TCA. The homogenate was filtered and 1.0 ml of filtrate was used for the assay.

Pyruvate

The pyruvate level was measured according to Friedemann and Haugen (1943). Homogenate (50 mg/ml, w/v) was prepared in 10% TCA. Sodium pyruvate was used as a standard.

Lactate

Lactate was estimated according to Barker and Summerson (1941), as modified by Huckabee (1961). Homogenate (50 mg/ml, w/v) was prepared in 10% cold (0°C) TCA. Sodium lactate was used as a standard.

Protease

Protease activity was estimated using the method of Moore and Stein (1954). Homogenate (50 mg/ml, w/v) was prepared in cold (0°C) distilled water. Optical density was measured at 570 nm with a spectrophotometer (Systronics, type 106). The enzyme activity was expressed in µmol protein activity $[\text{mg of protein}]^{-1}$ hour^{-1}.

Acid and alkaline phosphatases

Activities of acid and alkaline phosphatases were measured using the method of Bergmeyer (1967) as modified by Singh and Agarwal (1982). Tissue homogenate (2% w/v) was prepared in ice-cold 0.9% saline and centrifuged at 5000 g at 0°C for 15 min. Optical density was measured at 420 nm against a blank that was prepared simultaneously. The enzyme activity was expressed as mg of ρ-nitrophenol formed per 30 min per mg protein.

Lactic dehydrogenase

Lactic dehydrogenase (LDH) activity was measured according to Anonymous (1984). Homogenates (50 mg/ml, w/v) were prepared in 1 ml of 0.1 M phosphate buffer, pH 7.5, for 5 min in an ice bath. Enzyme activity was expressed as nmol of pyruvate reduced per min per mg protein.

Succinic dehydrogenase

Activity of succinic dehydrogenase (SDH) was measured using the method of Arrigoni and Singer (1962). Homogenate (50 mg/ml, w/v) was prepared in 1 ml of 0.5 M potassium phosphate buffers, pH 7.6, for 5 min in an ice bath. Optical density was measured at 600 nm. Enzyme activity was expressed as µmol dye reduced per min per mg protein.

Cytochrome oxidase

Cytochrome oxidase activity was measured according to the method of Cooperstein and Lazarow (1951). Homogenates (50 mg/ml, w/v) were prepared in 1 ml of 0.33 M phosphate buffer (pH 7.4) for 5 min in an ice bath. Enzyme activity was expressed as arbitrary units per min per (mg protein).

Acetylcholinesterase

Acetylcholinesterase was estimated by the method of Ellman *et al.* (1961) as adapted by Singh and Agarwal (1982) for snail tissue. Homogenates (50 mg/ml, w/v) were prepared in 0.1 M phosphate buffer in an ice bath. Optical density was measured at 412 nm using a spectrophotometer (Systronics, type 106). Enzyme activity was expressed as μmol of sulphohydryl group min^{-1} (mg protein)$^{-1}$.

RESULTS

The effects of sub-lethal (2.44 mg and 4.88 mg) exposure of the freshwater snail *Lymnaea acuminata* to aqueous extracts of the bark of the stem of *Croton tiglium* are given in Table 1. Sub-lethal doses of aqueous extracts for 96 h caused significant alterations in nitrogenous and carbohydrate metabolism in the nervous tissue, hepatopancreata, and ovotestes of these snails.

Effects on nitrogenous metabolism

After exposure to sub-lethal doses, total protein levels of nucleic acids (DNA and RNA) were significantly reduced, while free amino acid level was significantly enhanced in all of the body tissues of *Lymnaea acuminata* (Table 1). Activities of acid and alkaline phosphatases were significantly reduced, while protease activity was increased after the exposure.

Total protein levels in the nervous tissue, hepatopancreas, and ovotestis were reduced to 45%, 51%, and 42% of controls, respectively, after treatment with 4.88 mg of extracts. The DNA levels in the nervous tissue, hepatopancreas, and ovotestis were reduced to 39%, 48%, and 31% of controls, respectively, after treatment with 4.88 mg of extract. The RNA levels in the nervous tissue, hepatopancreas, and ovotestis were reduced to 47%, 54%, and 39% of controls, respectively, after treatment with 4.88 mg of extract. Total free amino acid levels in the nervous tissue, hepatopancreas, and ovotestis increased to 162%, 143%, and 165% of controls, respectively, after treatment with 4.88 mg of aqueous stem bark extracts.

Activities of acid phosphatase were inhibited to 46%, 75%, and 66% of controls after treatment with 4.88 mg of extracts respectively in nervous, hepatopancreas and ovotestis tissues. Activity of alkaline phosphatase was reduced to 35%, 40%, and 36% of controls after treatment with 4.88 mg of extracts respectively in nervous, hepatopancreas, and ovotestis tissue respectively. Protease activity was increased to 137%, 131%, and 133% of controls after treatment with 4.88 mg of extracts respectively in the nervous, hepatopancreas and ovotestis of snail *L. acuminata* (Table 1).

Effects on carbohydrate metabolism

Glycogen and pyruvate levels in all three body tissues were significantly reduced, while lactate level was significantly enhanced, after the exposure to sub-lethal doses (Table 1). Activities of lactic dehydrogenase (LDH), cytochrome oxidase, and acetylcholinesterase (AChE) were significantly reduced, while the activity level of succinic dehydrogenase (SDH) was increased after exposure.

Glycogen levels in the nervous tissue, hepatopancreas, and ovotestis were reduced to 36%, 46%, and 35% of controls, respectively, after treatment with 4.88 mg of extract. Pyruvate levels were reduced to 39%, 48%, and 37% of controls, respectively, after treatment with 4.88 mg of extract. Lactate levels were increased to 148%, 167%, and 173% of controls, respectively, after treatment with 4.88 mg of extract.

LDH activity in nervous, hepatopancreas and ovotestis tissue was reduced to 37%, 44%, and 39% of controls, respectively, after treatment with 4.88 mg of bark extract. Activity of cytochrome oxidase in these tissues was reduced to 45%, 49%, and 46% of controls, respectively after treatment with 4.88 mg of extracts. AChE activity was reduced to 46%, 42%, and 47% of controls, respectively, after treatment with 4.88 mg of bark extract and SDH activity was increased to 153%, 158%, and 151% of controls, respectively.

Withdrawal experiments

In the withdrawal experiments, there was nearly complete recovery of total protein, total free amino acid, lactate, cytochrome oxidase, succinic dehydrogenase, and protease and a partial recovery of glycogen, nucleic acids (DNA and RNA), pyruvate, lactic dehydrogenase, acetylcholinesterase, and activities of acid and alkaline phosphatase in the body tissues tested.

DISCUSSION

Exposure to aqueous extracts of the bark of the stem of *Croton tiglium* significantly altered the total protein, total free amino acid, glycogen, nucleic acids, pyruvate, and lactate levels and also significantly affected the activities of acetylcholinesterase, lactic dehydrogenase, cytochrome oxidase, protease, acid and alkaline phosphatases, and succinic dehydrogenase in nervous tissue, hepatopancreas, and ovotestis of the snail *Lymnaea acuminata.*

Gastropods exposed to stressful conditions generally use glycogen as their principal and immediate energy source (Goddard and Martin 1966). Protein is a stored energy source during chronic periods of stress. Animals exposed to sub-lethal concentrations of toxicant experience stress during the process of detoxification. The metabolic rates of the

Table 1. Changes in protein, free amino acids, nucleic acids (DNA and RNA) (μg/mg), protease (μmol of tyrosine equivalent per mg of protein per hour) acid and alkaline phosphatase (mg of ρ-nitrophenol formed per 30 min per mg protein), glycogen (μg/mg), pyruvate (μg/mg), lactate (μg/mg) LDH (nanomol of pyruvate reduced per min per mg protein) SDH (μmol dye reduced per min per mg protein) cytochrome oxidase (μmol of sulphohydryl group per min per mg protein), and AChE (arbitrary units per min per mg protein) in the nervous tissue (NT), hepatopancreas (HP) and ovotestis (OT) of the snail *Lymnaea acuminata* after 96 h exposure to sub-lethal doses of extracts of the stem of the bark of *Croton tiglium* and 7 days after withdrawal. Values are mean ± SE of six replicates.

	Tissues	Control	2.44 mg	4.88 mg	7 days after withdrawal
Protein	NT	64.5 ± 0.18	39.3 ± 0.25* (61)	29.0 ± 0.06 (45)	59.3 ± 0.24+ (92)
	HP	68.4 ± 3.61	41.7 ± 0.96* (61)	34.8 ± 0.84* (51)	64.9 ± 0.02+ (95)
	OT	71.3 ± 0.54	40.6 ± 0.89* (57)	29.9 ± 0.87* (42)	66.3 ± 0.41+ (93)
Amino acids	NT	33.4 ± 1.12	50.5 ± 1.18* (151)	54.5 ± 0.32* (162)	35.7 ± 0.04+ (107)
	HP	28.6 ± 1.53	35.0 ± 0.42* (122)	41.6 ± 1.09* (143)	29.4 ± 0.22+ (103)
	OT	36.8 ± 0.77	56.7 ± 0.18* (154)	60.7 ± 0.17* (165)	38.3 ± 0.34+ (105)
DNA	NT	72.83 ± 0.33	43.6 ± 0.34* (60)	28.4 ± 0.35* (39)	69.18 ± 0.84+ (95)
	HP	70.83 ± 0.33	49.50 ± 0.37* (70)	33.91 ± 0.32* (48)	69.41 ± 1.12+ (98)
	OT	73.50 ± 0.88	40.33 ± 0.23* (54)	23.50 ± 0.54* (31)	67.62 ± 0.71+ (92)
RNA	NT	61.51 ± 0.47	33.35 ± 0.41* (64)	28.91 ± 0.35* (47)	59.04 ± 0.98+ (96)
	HP	59.10 ± 0.87	43.14 ± 0.81* (73)	39.92 ± 0.25* (54)	38.50 ± 0.78+ (99)
	OT	64.16 ± 0.44	36.00 ± 3.49* (56)	25.33 ± 0.61* (39)	59.66 ± 0.81+ (93)
Protease	NT	0.298 ± 0.054	0.360 ± 0.051* (121)	0.408 ± 0.065* (137)	0.283 ± 0.046+ (95)
	HP	0.318 ± 0.001	0.375 ± 0.02* (118)	0.416 ± 0.002* (131)	0.289 ± 0.062+ (91)
	OT	0.324 ± 0.009	0.385 ± 0.006* (199)	0.430 ± 0.006* (133)	0.314 ± 0.028+ (97)
Acid phosphatase	NT	0.188 ± 0.009	0.092 ± 0.009* (49)	0.086 ± 0.008* (46)	0.172 ± 0.007+ (92)
	HP	0.185 ± 0.008	0.098 ± 0.007* (53)	0.138 ± 0.006* (75)	0.174 ± 0.009+ (75)
	OT	0.182 ± 0.008	0.083 ± 0.0009* (46)	0.120 ± 0.0007* (66)	0.174 ± 0.0005+ (96)
Alkaline phosphatase	NT	0.385 ± 0.025	0.150 ± 0.0004* (39)	0.134 ± 0.0003* (35)	0.350 ± 0.0018+ (91)
	HP	0.355 ± 0.006	0.156 ± 0.0007* (44)	0.142 ± 0.0003* (40)	0.333 ± 0.0012+ (94)
	OT	0.379 ± 0.009	0.140 ± 0.0001* (37)	0.136 ± 0.0002* (36)	0.352 ± 0.0013+ (93)
Glycogen	NT	7.8 ± 0.02	4.5 ± 0.04* (58)	2.8 ± 0.02* (36)	7.4 ± 0.03+ (95)
	HP	7.2 ± 0.10	4.8 ± 0.02* (67)	3.3 ± 0.02* (46)	6.9 ± 0.02+ (96)
	OT	7.9 ± 0.10	4.5 ± 0.04* (57)	2.7 ± 0.02* (35)	7.3 ± 0.04+ (93)
Pyruvate	NT	0.678 ± 0.02	0.289 ± 0.20* (43)	0.271 ± 0.11* (39)	0.610 ± 0.27+ (90)
	HP	0.699 ± 0.003	0.356 ± 0.07* (51)	0.335 ± 0.07* (48)	0.643 ± 0.08+ (92)
	OT	0.673 ± 0.02	0.275 ± 0.21* (41)	0.249 ± 0.12* (37)	0.612 ± 0.03+ (91)
Lactate	NT	2.01 ± 0.06	2.92 ± 0.14* (145)	2.97 ± 0.14* (148)	2.13 ± 0.04+ (106)
	HP	2.27 ± 0.04	3.54 ± 0.06* (156)	3.67 ± 0.02* (167)	2.46 ± 0.05+ (108)
	OT	2.08 ± 0.01	2.97 ± 0.03* (143)	3.59 ± 0.02* (173)	2.30 ± 0.03+ (111)
LDH	NT	0.069 ± 0.004	0.048 ± 0.004* (69)	0.025 ± 0.002* (37)	0.062 ± 0.003+ (90)
	HP	0.082 ± 0.003	0.059 ± 0.001* (73)	0.036 ± 0.003* (44)	0.076 ± 0.004+ (92)
	OT	0.065 ± 0.004	0.046 ± 0.002* (71)	0.025 ± 0.007* (39)	0.061 ± 0.003+ (94)
SDH	NT	17.01 ± 0.12	22.78 ± 0.13* (134)	26.02 ± 0.18* (153)	18.71 ± 0.84+ (110)
	HP	14.50 ± 0.14	20.01 ± 0.17* (138)	22.91 ± 0.02* (158)	15.51 ± 0.18+ (107)
	OT	14.50 ± 0.14	19.14 ± 0.31* (132)	21.89 ± 0.32* (151)	16.53 ± 0.20+ (114)
Cytochrome oxidase	NT	21.02 ± 0.14	11.77 ± 0.17* (56)	9.45 ± 0.18* (45)	19.12 ± 0.04+ (91)
	HP	14.62 ± 0.12	8.77 ± 0.18* (60)	7.16 ± 0.13* (49)	13.59 ± 0.12+ (93)
	OT	19.01 ± 0.12	10.83 ± 0.17* (57)	8.74 ± 0.18* (46)	18.05 ± 0.02+ (95)
AChE	NT	0.068 ± 0.007	0.038 ± 0.002* (55)	0.031 ± 0.002* (46)	0.061 ± 0.007+ (90)
	HP	0.081 ± 0.006	0.048 ± 0.001* (60)	0.034 ± 0.001* (42)	0.076 ± 0.002+ (94)
	OT	0.067 ± 0.006	0.037 ± 0.002* (56)	0.031 ± 0.003* (47)	0.060 ± 0.007+ (91)

Values in parentheses are % level. Control taken as 100%. Data were analyzed by Student t test.*, Significant ($P<0.05$) when treated groups were compared withcontrol. +, Significant ($P<0.05$) when Students t test was applied between treated and withdrawal groups.

fish *Mystus vittatus* (Bloch, 1797) reared at different concentrations of toxicant can be greater than that of animals reared in freshwater (Aranachalem *et al.* 1980). The depletion of protein fraction in nervous tissue, hepatopancreas, and ovotestis of *Lymnaea acuminata* may have been due to their degradation and possible utilization of degraded products for metabolic purposes. The increase in the level of free amino acids was probably the result of the breakdown of protein for energy requirements and impaired incorporation of amino acids in protein synthesis, but it also could be attributed to the reduced use of amino acids and their involvement in the maintenance of an acid-base balance. Stress conditions induce the transamination pathway. Inhibition of DNA synthesis might affect levels of protein and amino acids by decreasing the level of RNA in the machinery for protein synthesis (Nordenkjold *et al.* 1979). Euphorbiales are potential inhibitors (Singh *et al.* 1996) of DNA synthesis, causing a reduction of RNA levels and consequently affecting protein synthesis and amino acid levels, as shown by our results.

The enzyme protease functions in hydrolysing proteins to free amino acids and small peptides. The increase in the protease activity corroborates with the enhancement in the levels of free amino acids in the three tissues, the formation of which might be the result of protein hydrolysis, suggesting stimulation during toxic stress. A similar trend in protease activity has been reported by several workers in different animals (*Tilapia mossambica*, *Pila globosa* and various mammals) (Millward 1970, Siva Prasada Rao 1980, Sivaiah 1980, Kabeer *et al.* 1984). The enzyme aminotransaminase provides a link between carbohydrate and protein metabolism because they interconvert metabolites such as α-ketogluterate, pyruvate, and oxaloacetate on the one hand and alanine, aspartate, and glutamate on the other hand. Aminotransaminase plays an important role in the utilization of amino acids for the oxidation and/or for gluconeogenesis. An increase in aminotransaminase activity indicated the utilization of amino acids for the formation of oxaloacetate, α-ketogluterate, and pyruvate to meet energy demands. This is supported by the observation of Malla Reddy and Bashamohiden (1995) in the metabolism of selected tissues of the fish *Cyprinus caprio* exposed to sub-lethal concentrations of the toxicant cypermethrin. Because the plant used in this study may have some antiphosphatase activity, the reduction in protein level may be due to the inhibition of alkaline phosphatase activity, as the latter plays an important role in protein synthesis (Pilo *et al.* 1972).

Anticholinesterase compounds are known to inhibit mitochondrial cytochrome oxidase, which is a terminal enzyme of the electron transport chain. Inhibition of cytochrome oxidase by plant moieties indicates that euphorbs might have a profound impact on oxidative metabolism. Decrease in cytochrome oxidase might either be the result of reduced availability of oxygen, which in turn reduces the capacity of the electron transport system to produce ATP, or could be due to the direct impact of the active moiety, such as the function of cytochrome oxidase in the electron transport system (Sambasiva Rao 1999). Because euphorbs are anticholinesterase inhibitors, they may affect the Kreb's cycle by diminishing the rate of the electron transport system and oxidative phosphorylation, reducing the synthesis of ATP.

Reduction of glycogen level is believed to result from the greater stress the organs experienced during the detoxification of active moieties and their metabolites. Euphorbiales inhibit acetylcholinesterase activity, which results in an increase of acetylcholinesterase convert (Singh *et al.* 1996). An increased level of acetylcholine has been shown to enhance the secretion of catacholine (Nilsson *et al.* 1976), which may bring about glucogenolysis. Thus glycogenolysis seems to result from increased secretion of catecholine due to stress (Singh and Agarwal 1993).

The increase in lactate also suggests a shift towards anaerobiosis as a consequence of hypoxia leading to respiratory distress (Siva Prasada Rao 1980). Development of such internal hypoxic conditions may ultimately be responsible for the shift to the less efficient anaerobic metabolism, evidenced by the change in lactate content observed during this study.

The present study also demonstrates that the extracts of *Croton tiglium* have stronger molluscicidal activity than any of the prevalent synthetic molluscicides such as carbamate, organophosphate, and synthetic pyrethroids. The 24 h LC_{50} of mexacarbate (3.5 ppm), aldicarbe (30 ppm), farmothion (27 ppm), cypermethrin (2.5 ppm), permethrin (0.82 ppm), and fenvalerate (2.5 ppm) for the freshwater snail *Lymnaea acuminata* (Singh and Agarwal 1981, 1991, Sahay *et al.* 1991) is higher than that of the croton extract (2.44 mg/l), which is about 20 times stronger than the LC_{50} of the standard molluscicide niclosamide LC_{50} (11.8 mg/l) (Singh and Agarwal 1988).

In conclusion, extracts of the bark of the stem of *Croton tiglium* are a potential source of molluscicides. Plant products are less expensive, easily available, easily soluble in water, and have low toxicity to non-target animals (Yadav and Singh 2001). We therefore believe that these plant extracts may eventually be of great value for the control of aquatic organisms.

LITERATURE CITED

Anonymous. 1984. Sigma diagnostics TM: Lactic dehydrogenase (quantitative, colorimetric determination in serum, urine and cerebrospinal fluid) at 400-450 nm. Procedure No. 500. St. Louis, U.S.A. Sigma. Aldrich-India (Survey 31/1), Sitharamapalaya, Mahadevapur Banglore India.

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1980. *Standard Methods for the Examination of Water and Wastes Water*, 16th Ed. American Public Health Association, New York.

Aranachalam, S., K. Jayalakshami, and S. Abooker. 1980. Toxic and sub-lethal effects of carbaryl on a freshwater catfish *Mystus vittatus. Archives of Environmental Contamination and Toxicology* **9**: 307-316.

Arrigoni, O. and T. P. Singer. 1962. Limitation of the phenozine methosulphate assay for succinic and related dehydrogenase. *Nature* **193**: 1256-1258.

Barker, S. B. and W. H. Summerson. 1941. The colorimetric determination of lactic acid in biological materials. *Journal of Biological Chemistry* **138**: 535-542.

Bergmeyer, U. H. 1967. *Methods of Enzymatic Analysis*. Academic Press, New York.

Cooperstein, S. J. and M. Lazarow. 1951. A microspectrophotometric method for the determination of cytochrome oxidase. *Journal of Biological Chemistry* **189**: 665-670.

Ellman, G. L., K. D. Courtney, V. J. R. Andres, and R. M. Featherstone. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology* **7**: 88-95.

Friedemann, T. E. and G. F. Haugen. 1943. Pyruvic acid. I. Collection of blood for the determination of pyruvic acid and lactic acid. *Journal of Biological Chemistry* **144**: 67-77.

Goddard, C. K. and A. W. Martin. 1966. Carbohydrate metabolism. *In*: K. M. Wilbur and C. M. Yonge, eds., *Physiology of Mollusca*, Vol. 2. Academic Press, New York. Pp. 275-308.

Huckabee, W. E. 1961. Blood analysis, determination of lactic acid. *In:* B. L Oser, ed., *Hawk's Physiological Chemistry*, 14th Ed. Tata McGraw Hill, New Delhi. P. 1103.

Kabeer, A., I. Sahib, R. K. Siva Prasad, R. K. R. Sambasiva, and K. V. Ramana Rao. 1984. Sub-lethal toxicity of malathion on the protease and free amino acid composition of the liver of the teleost *Tilapia mossambica* (Peters). *Toxicology Letter* **20**: 59-62.

Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with folin phenol reagent. *Journal of Biological Chemistry* **193**: 265-275.

Mahendru, V. K. and R. A. Agarwal. 1982. Changes induced by phorate in the carbohydrate metabolism of the snail *Lymnaea acuminata. Pesticides Science* **13**: 611-616.

Malla Reddy, P. and M. Bashamohiden. 1995. Alteration in protein metabolism in selected tissue of the fish *Cyprinus caprio* during sub-lethal concentration of cypermethrin. *Bulletin of Environmental Contamination and Toxicology* **36**: 183-190.

Millward, D. J. 1970. Protein turnover in skeletal muscle II. The effect of starvation and protein free diet on the synthesis and catabolism of skeletal muscle protein in comparison to liver. *Clinical Science* **39**: 591-603.

Moore, S. and W. H. Stein. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *Journal of Biological Chemistry* **211**: 907-913.

Nilsson, S., R. Abrahamsson, and D. J. Grove. 1976. Sympathetic nervous control of adrenalin release from the head kidney of cod *Gardus morhua. Comparative Biochemistry Physiology* (C)**55**:123-127.

Nordenkjold, M., J. Soderhall, and P. Moldeus. 1979. Studies on DNA strand breaks induced in human fibroblasts by chemical mutagens and carcinogens. *Mutation Research* **63**: 393-400.

Pilo, B., M. V. Asnani, and R. V. Shah. 1972. Studies on wound healing and repair in pigeon. III. Histochemical studies on acid alkaline phosphatase activity during the process. *Journal of Animal Physiology* **19**: 205-212.

Sahay, N., D. K. Singh, and R. A. Agarwal. 1991. Synergistic effect of piperonyl butoxide on the toxicity of synthetic pyrethroids in the snail *Lymnaea acuminata. Journal of Medical and Applied Malacology* **3**: 107-111.

Sambasiva Rao, K. R. S. 1999. *Pesticide Impact on Fish Metabolism*. Discovery Publishing House, New Delhi, India.

Schneider, W. C. 1957. Determination of nucleic acid by pentose analysis. *In:* S. P Calowick and N. O. Kaplon, eds., *Enzymology*, Academic Press, New York. Pp. 680-683.

Singh, A. and R. A. Agarwal. 1988. Possibility of using latex of euphorbiales for snail control. *The Science of the Total Environmental* **77**: 231-236.

Singh, A. and R. A. Agarwal. 1993. Effect of Cypermethrin on lactate and succinic dehydrogenase and cytochrome oxidases of snail and fish. *Bulletin of Environmental Contamination and Toxicology* **51**: 445-452.

Singh, A., D. K. Singh, T. N. Mishra, and R. A. Agarwal. 1996. Molluscicides of plant origin. *Biological Agriculture Horticulture* **13**: 205-252.

Singh, D. K. and R. A. Agarwal. 1982. Synergistic effect of sulfoxide with carbaryl on in vivo acetylcholinesterase activity and carbohydrate metabolism of the snail *Lymnaea acuminata. Acta Hydrochimica et Hydrobiologica* **14**: 421-427.

Singh, D. K. and R. A. Agarwal. 1991. Action sites of cypermethrin, a synthetic pyrethroid in the snail *Lymnaea acuminata. Acta Hydrochimica et Hydrobiologica* **19**: 425-430.

Singh, O. and R. A. Agarwal. 1981. Toxicity of certain pesticides to two economic species of snails in Northern India. *Journal of Economic Entomology* **74**: 568-571.

Siva Prasada Rao, K. 1980. *Studies on Some Aspects of Metabolic Changes with Emphasis on Carbohydrate Utility in the Cell-Free Systems of the Teleost* Tilapia mossambica *(Peters) under Methyl Parathion Exposure*. Ph.D. Dissertation, Sri Venkateswara Universities, Tirupati, India.

Sivaiah, S. 1980. *Studies on Some Aspects of Physiology and Enzymatic Change in Cell Free System of the Snail* Pila globosa *(Swaimson) Subjected to Malathion Exposure*. Ph.D. Dissertation, Sri Venkateswara Universities, Tirupati, India.

Sokal, R. R. and F. J. Rohlf. 1973. *Introduction to Biostatistics*. M. N. Freeman, San Francisco.

Spices, J. R. 1957. Colorimetric procedure for amino acids. *In:* S. P. Calowick and N. O. Kalpan, eds., *Methods in Enzymology*. Academic Press, New York. P. 468.

Uphof, J. C. T. 1968. *Dictionary of Economic Plants*, 2nd Ed. Verolog Van J. Gramer, Lehre, Germany.

Van Der Vies, J. 1954. Two methods for the determination of glycogen in liver. *Biochemistry Journal* **57**: 410-416.

Yadav, R. P. and A. Singh. 2001. Environmentally safe molluscicides from two common euphorbiales. *Iberus* **19**: 65-73.

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Histology of selected regions of the alimentary system of *Strombus gigas* Linnaeus, 1758 (Caenogastropoda: Strombidae)

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Abstract: The histology of the alimentary system of *Strombus gigas* was examined, focusing on the epithelial tissue and subepithelial tissue of the mouth, style sac, digestive gland, and anus. These four structures were chosen because of their functions: (1) ingestion of food (mouth); (2) digestion, absorption, and storage of nutrients (style sac and digestive gland); and (3) elimination of feces (anus). The mouth had a pseudostratified epithelium; the style sac had simple cuboidal and stratified epithelia. The digestive gland was glandular and the anus had columnar and pseudostratified epithelia. The cilia on the columnar epithelium were dispersed; the cilia on the cuboidal epithelium formed tufts. The glandular epithelium was arranged in acini. Fibrous connective tissue was found in the mouth and in the anus. Loose connective tissue occurred in the style sac, digestive gland, and anus. Interwoven in the fibrous connective tissue of the mouth and anus were muscle fibers, connective tissue fibers, and blood cells. Two types of blood cells occurred in the subepithelial connective tissue. Granular cells that were 2-4 μm in diameter occurred in the mouth, style sac, and anus. The style sac also contained blood cells that were 6 μm in diameter, and had very large nuclei (up to 4 μm in diameter). The tissues described in this work are similar to those described for some bivalves and other gastropods.

Key Words: digestive system, digestive gland, style sac, Gastropoda, Queen conch.

The Queen conch *Strombus gigas* Linnaeus, 1758 (Caenogastropoda, Strombidae) is the largest of five species of *Strombus* occurring in the Caribbean region (Abbott 1986). It is a resource of commercial importance to several Caribbean countries. Fishing pressure has reduced its populations throughout its range (Creswell 1994, Stoner 1997) to the point that, since 1992, it has been included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and since 1994 was classified in the Red List of Threatened Animals of the International Union for Conservation of Nature and Natural Resources (IUCN).

Strombus gigas has been studied since the 1960s (Stoner 1997), resulting in more than 600 scientific articles (Darcy 1981, Acosta 1994, Ray-Culp 2003). Even so, there have been few studies on its histology, and these have focused on the reproductive system (Egan 1985, Buckland 1989, Reed 1993, 1995a, 1995b, Aldana-Aranda *et al.* 2003).

Queen conchs are marine herbivores, whose diet consists mainly of epiphytic algae, filamentous algae, and detritus (Randall 1964, Brownell and Stevely 1981, Stoner and Waite 1991). Little (1965) illustrated the internal anatomy of *Strombus gigas*, focusing on parts of the alimentary tract. Horiuchi and Lane (1965, 1966) described the cellulase and carbohydrase activities of the crystalline style and digestive gland of *S. gigas* and Little (1967) examined the ionic regulation of body fluids in the alimentary system of *S. gigas*.

The histology of the alimentary system is needed in studies related to acquisition of food, digestion, and absorption. The objective of this study was to describe the epithelial tissue and subepithelial tissue of selected regions of the alimentary system of *Strombus gigas* based on their functions, including the mouth (ingestion), the style sac and digestive gland (digestion, absorption, and storage of nutrients), and the anus (elimination of feces).

MATERIALS AND METHODS

Three male and three female individuals of *Strombus gigas* with shell lengths of 228 mm (± 20 mm) and lip thickness of 16 mm (± 1.8 mm) were collected from Alacranes Reef (22°22′96″N; 89°41′03″W), North of Yucatan Peninsula, Mexico, in June 2001.

Each whole animal was measured and weighed before extraction from its shell. Extraction of soft parts was achieved by boring a hole on the third turn of the spire, detaching the columellar muscle from the columella. Samples of 1 cm^3 from the mouth, style sac, digestive gland, and anus (Fig. 1) were fixed in Davidson's fixative (Elston 1990) for four days; rinsed in 70% ethanol; dehydrated in 70%, 96% and 100% ethanol; cleared in benzene, and embedded in Paraplast® tissue embedding medium (m.p.

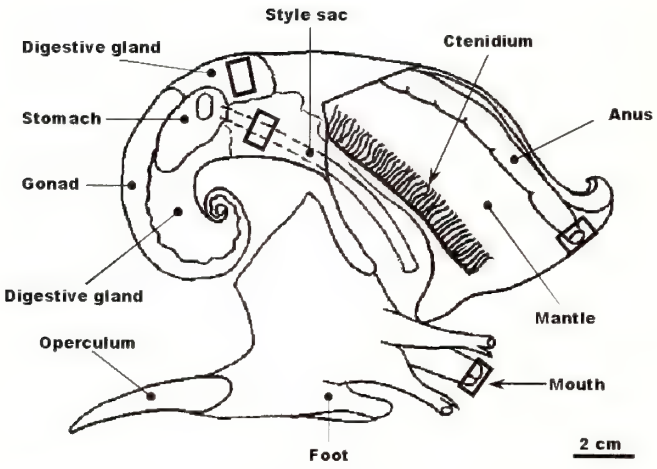


Figure 1. Schematic drawing of a female *Strombus gigas* viewed from the right and removed from its shell with the mantle cavity opened on its right side, showing the internal wall of the mantle. Rectangles indicate the sites where the samples were taken.

56°C). Serial sections of 6 μm were cut with a rotary microtome, Microm HM340E, and mounted on glass slides with Meyer's albumin. Harris's Hematoxylin and Eosin, regressive method, was used for staining (Howard and Smith 1983). In addition, Masson's trichrome stain was used to identify connective tissue (Gabe 1968). Blood cells were recognized using established descriptions by García-Cerruti (1977). The slides were examined at magnifications of 30×, 100x, 400x and 1000x using a Carl Zeiss MC73A light microscope. Sections were photographed with a Sony color video camera of high resolution, model CCD-IRIS, mounted on the microscope. Each digital image was stored in a computer in graphic format to produce the figures of this manuscript.

RESULTS

Mouth

The mouth is located in the apex of the proboscis and consists of two lips of epithelial tissue and subepithelial connective tissue.

The epidermis of the mouth consisted of non-ciliated pseudostratified epithelium forming folds (Figs. 2-3). This epithelium was composed of a single layer of columnar cells, but not all cells reached the external lamina. This epithelium had an external lamina 5-12 μm thick and a basal lamina 2 μm thick. The columnar cells measured 60-80 μm high, with granular elongated nuclei 12-14 μm in length and 2-4 μm wide. The position of the nucleus within the cell was highly variable, forming a zone up to 45 μm high in the central region of each epithelial cell. This zone occupied nearly 60% of the height of the epithelium, with only 10-16 μm of cytoplasm towards the apical and basal surfaces of the cell.

The subepithelial connective tissue was fibrous, with shiny fibers 2 μm thick in section, and muscle fibers 0.8 μm thick, interwoven in the subepithelial connective tissue. Granular blood cells, 2-3 μm in diameter, were scattered in the connective tissue (Figs. 2 and 3 and Table 1).

Style sac

The style sac was embedded in the visceral mass (Fig. 4). The wall adjacent to the visceral mass was covered by a stratified epithelium composed by several layers of cells that were disorganized and of irregular shape. Theses cells were characterized by oval nuclei 2-4 μm in diameter (Fig. 5). The style sac consisted of loose, hyaline connective tissue interwoven by thin fibers. Ciliated cuboidal epithelial cells 16-18 μm in height with granular rounded nuclei 6 μm in diameter lined the lumen. The short cilia of these cells formed a dense mat. The cilia measured 18-22 μm in length. Between the hyaline connective tissue and the ciliated cuboidal epithelium a layer of fibers was observed (Fig. 6; Table 1).

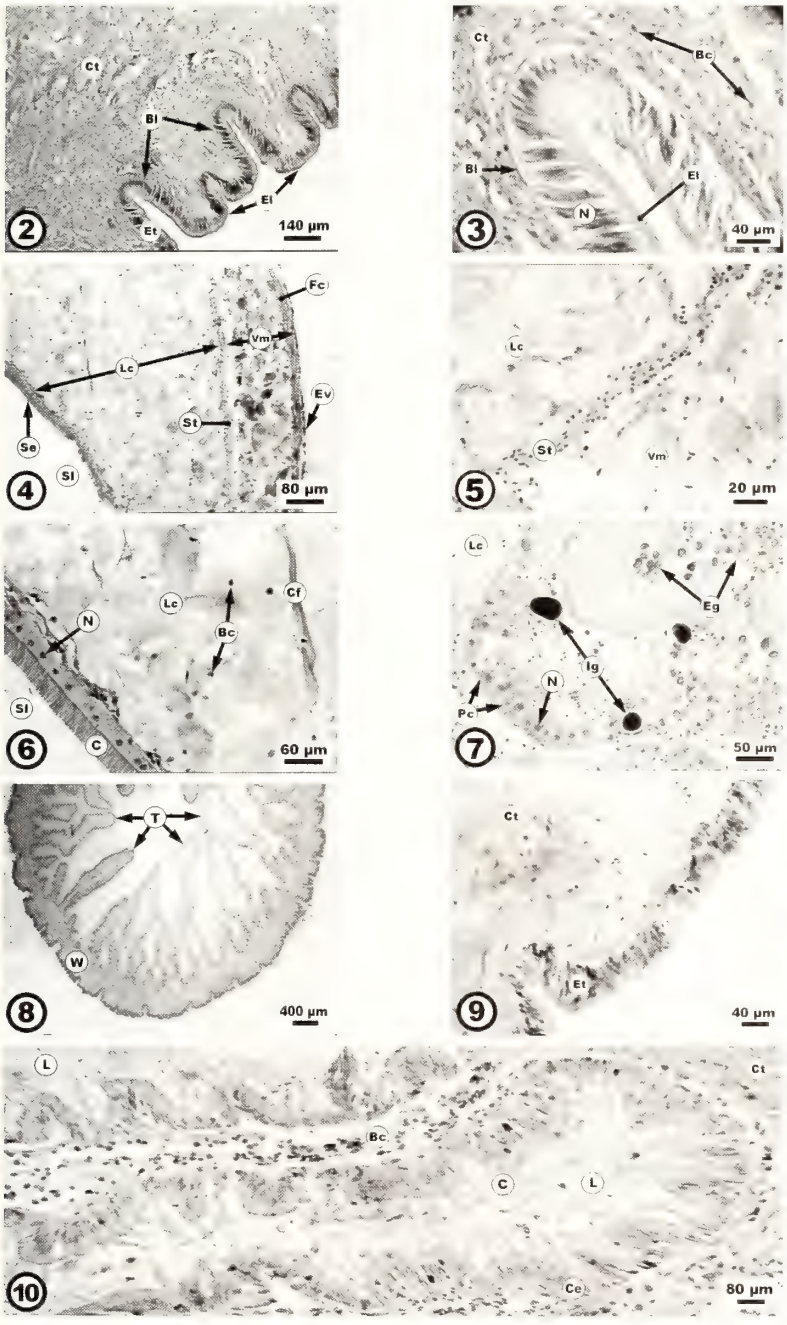
Digestive gland

The digestive gland was a compound glandular structure of acini embedded in a hyaline, loose connective tissue that was interwoven by thin fibers (Fig. 7). The glandular acini were formed by truncated pyramidal cells that contained granules. These cells were 10-16 μm wide at their bases and 4-6 μm wide at their apices. Each nucleus was apical and granular, with a diameter of 4 μm. These cells contained other intracellular granules 14-20 μm in diameter, which were surrounded by a clear halo 1-3 μm wide. In the lumen of the glandular acini were extracellular granules 2-8 μm in diameter (Table 1).

Anus

The internal lumen of the anus was lined by the folds of the typhlosoles (Fig. 8). The anal wall was covered by non-ciliated pseudostratified columnar epithelium. Their nuclei were elongated, granular, and 4-6 μm in diameter. Connective tissue fibers 2-3 μm thick interwoven by muscle fibers filled the anal wall, which had a thickness of 275-450 μm. Within the connective tissue were granular, oval blood cells that were 2-3 μm in diameter (Fig. 9).

The epithelium of the typhlosoles was columnar and ciliated, formed by cells 30-40 μm high whose nuclei were oval, 2-8 μm in diameter, and located basally. The cilia measured 16-20 μm in length, forming tufts. Each fold contained a thin layer of fibrous connective tissue and loose connective tissue. Blood cells in the typhlosoles were rounded, up to 6 μm in diameter, and characterized by a tenuous cytoplasm and spherical nuclei 4 μm in diameter (Fig. 10; Table 1).



Figures 2-10. Fig. 2, Digital image of a histological section through of the mouth of *Strombus gigas* stained with Harris's Hematoxylin and Eosin. Bl, basal lamina of epithelium; Ct, fibrous connective tissue; El, external lamina of epithelium; Et, pseudostratified columnar epithelial tissue. Fig. 3, Digital image of a histological section of the undulating epithelium of the mouth of *Strombus gigas* stained with Harris's Hematoxylin and Eosin. Bc, blood cells; Bl, basal lamina; Ct, fibrous connective tissue; El, external lamina; N, nuclei. Fig. 4, Digital image of a histological section of the style sac and visceral mass of *Strombus gigas* stained with Masson's trichrome. Ev, external surface of the visceral mass; Fc, fibrous connective tissue; Lc, loose connective tissue of the style sac; Se, epithelium lining the lumen of the style sac; Sl, lumen of the style sac; St, stratified epithelium; Vm, subepithelial connective tissue of the visceral mass. Fig. 5, Digital image of a histological section of the adjacent wall of the style sac and visceral mass of *Strombus gigas* stained with Masson's trichrome. Lc, loose connective tissue of the style sac; St, stratified epithelium; Vm, subepithelial connective tissue of the visceral mass. Fig. 6, Digital image of a histological section of the cuboidal epithelium lining the lumen of the style sac of *Strombus gigas* stained with Masson's trichrome. Bc, blood cells; C, cilia of cuboidal epithelium; Cf; connective tissue fiber; Lc, loose connective tissue; N, nucleus of cuboidal epithelium; Sl, lumen of the style sac. Fig. 7, Digital image of a histological section of the digestive gland of *Strombus gigas* stained with Harris's Hematoxylin and Eosin. Eg, extracellular granules; Ig, intracellular granules; Lc, loose connective tissue; N, nucleus of a pyramidal cell; Pc, pyramidal cells. Fig. 8, Digital image of a transverse histological section through of the anus of *Strombus gigas* showing the typhlosoles stained with Harris's Hematoxylin and Eosin. T, typhosoles; W, anal wall. Fig. 9, Digital image of a histological section of the anal wall of *Strombus gigas* stained with Harris's Hematoxylin and Eosin. Ct, fibrous connective tissue; Et, pseudostratified columnar epithelial tissue. Fig. 10, Digital image of a histological section of the typhlosoles of *Strombus gigas* stained with Harris's Hematoxylin and Eosin. Bc, Blood cells among connective tissue; C, cilia; Ce; columnar epithelium; Ct, fibrous connective tissue; L, lumen.

DISCUSSION

Ward (1966), Harris *et al.* (1998), and Voltzow (1994) described variations of the epithelial tissue for the alimentary system of *Fissurella barbadensis* Gmelin, 1791, *Haliotis laevigata* Donovan, 1808, and of several others species of "prosobranchs," respectively. These authors stated that the variations of the epithelia could be the result of different functions: protection, trans-cellular transportation, secretion, absorption, and lubrication. In this study of the alimentary system of *Strombus gigas* a variety of different epithelial tissues were observed.

The cilia of the epithelial tissues of the digestive system of *Strombus gigas* were highly variable. Cilia of the cuboidal epithelium lining the lumen of the style sac were numerous, forming a dense mat, and were taller than the cells themselves (Fig. 6). The cilia of the anus were dispersed and were usually shorter than the heights of the cells (Fig. 10). Warren (1959) proposed that this clustered arrangement increases the surface of the cells. The cilia lining the style sac are

Table 1. Summary of the composition of epithelial tissue of four selected regions of the alimentary system of *Strombus gigas.*

		Cell		Cilia		Nucleus		
Structure	Type of epithelium	Shape	Height µm	Length µm	Cluster	Position in the cell	Size µm	Feature
Mouth	pseudostratified	columnar	60-80	none	none	central	4-14	elongated
	stratified	irregular		none	none	none	2-4	oval
Style sac	simple cuboidal	cuboidal	16-18	18-22	grouped	central	6	rounded, granulose
Digestive gland	glandular	truncated pyramidal		none	none	mid-apical	4	rounded, granulose
Anus (typhlosole)	simple columnar	columnar	30-40	16-20	separated	basal	2-8	oval

associated with the movement of the style and food particles (Fretter and Graham 1962, Hughes 1986), whereas in the typhlosoles of the anus they are involved in osmoregulation and with the discharge of fecal matter.

Loose connective tissue is characteristic of structures that do not require protection and is involved in nutrition and storage of proteins and fats; fibrous connective tissue is characteristic of structures that need support and distension (Gartner and Hiatt 1997). In gastropods, subepithelial connective tissue provides support between the epidermis and the organs (Bairati *et al.* 2001). In this study, loose connective tissue was observed in the style sac and digestive gland and fibrous connective tissue was observed in the mouth and anal wall. Voltzow (1990) described the arrangement of the muscle fibers and connective tissue in the foot of *Busycon contrarium* Conrad, 1840 and *Haliotis kamtschatkana* Jonas, 1845 as individual muscle fibers embedded in a dense matrix of connective tissue. Thompson *et al.* (1998) described the arrangement of the columellar muscle of *Calliostoma euglyptum* Adams, 1854, *Littorina littorea* Linnaeus, 1758, and *Ilyanassa obsoleta* Say, 1822 as muscle and connective tissue fibers. Greene and Kohn (1989) described, in the proboscis of *Conus catus* Hwass, 1792, very few muscle bundles that are gradually replaced by connective tissue, which allows for extension of the proboscis. In this study we observed few muscle fibers interwoven in the subepithelial connective tissue of the mouth and anus of *Strombus gigas.*

Two morphologically different blood cells were observed in the subepithelial connective tissue of the alimentary system of *Strombus gigas.* The blood cells at the mouth and the style sac were granular amoebocytes; macrophages were observed in the anus. Sminia *et al.* (1983) stated that hemolymph and connective tissue serve as large reservoirs of blood cells. García-Cerruti (1977) found several types of blood cells in the hemolymph of *S. gigas.* Little (1965) and García-Cerruti (1977) named these cells amoebocytes. García-Cerruti (1977) categorized amoebocytes based on the different colors of the cytoplasm based on staining with hemotoxylin-eosin and the size relation between the cell and the nucleus. Sminia *et al.* (1983) and van der Knaap *et al.* (1993) stated that gastropods possess one basic type of amoebocyte, which expresses various morphological forms. These blood cells pass through a series of differentiations from round (young cells) to readily spreading (mature cells). Blood cells located in the style sac of *S. gigas* were most likely associated with transport and digestion, while macrophages at the mouth and anus could have a protective function, given their close relation to the exterior.

The style sac is a characteristic structure of some molluscs that have crystalline styles, such as herbivorous "prosobranchs," lamellibranchs, and eulamellibranchs (Salvini-Plawen 1988). The ciliated cuboidal epithelium found in the style sac of *Strombus gigas* was similar to those reported for *Batillaria zonalis* Bruguière, 1792, *Cerithidea californica* Haldeman, 1840 (Driscoll 1972), and *Telescopium telescopium* Linnaeus, 1758 (Alexander and Rae 1974). Warren (1959), Driscoll (1972). Hughes (1986) proposed that ciliary action rotates the crystalline style and drives particles toward the stomach, the digestive gland, and the intestinal cavity. Little (1967) proposed that the food is transported in the stomach of *S. gigas* by secretions from the digestive gland and by enzymes liberated from the crystalline style. He indicated that the ciliary action in the stomach moves small particles towards the digestive gland, where these particles are absorbed and digested intracellularly, and that rejected matter goes to the intestine and rectum.

Owen (1966) and Morton (1979) described the digestive gland of gastropods as a structure composed of numerous tubules that are lined by at least two types of cells. We observed only pyramidal cells in the digestive gland of *Strombus gigas.* Hughes (1986) described these cells as conical with broad bases and narrow tips reaching the lumen. Kress *et al.* (1994) described them as triangular with an apical irregular border for *Runcina coronata* Quatrefages, 1844 and *Runcina ferruginea* Kress, 1977. These authors mentioned that this shape is a characteristic of secretory cells. Hughes (1986) indicated that the digestive gland stores minerals used in shell formation. Checa and Jiménez-Jiménez (1998) point to several gastropods minerals used in calcification and growth of the operculum. In this study of

the digestive gland of *Strombus gigas* we observed the characteristics describe by previous authors.

The morphology and histology of the anus of *Strombus gigas* is similar to that of other molluscs. Jegla and Greenberg (1968) classified the bivalve rectum according to (1) shape of the lumen, (2) thickness of the wall of the rectum, and (3) composition and arrangement of tissue elements. Ward (1966) described large longitudinal folds in the anus of the gastropod *Fissurella barbadensis*, with cells 15 to 20 μm tall, with cilia 5 μm long, and with prominent basal granules. This epithelium is surrounded by connective tissue. Harris *et al.* (1998) distinguished columnar ciliated cells beginning in the final region of the intestine to the anus of the gastropod *Haliotis laevigata*. Dimitriadis (2001) considered that the projections of folds into the lumen of the anus of gastropods are major typhlosoles and ciliated ridges that function in the transport of particulate material during the discharge of feces. The shape of the lumen of the anus of *S. gigas* suggests the presence of typhlosoles described by previous authors as a projection into the lumen of connective tissue with a covering of ciliated epithelium. Jegla and Greenberg (1968) suggest the presence of typhlosoles might function in osmoregulation.

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LITERATURE CITED

Abbott, R. T. 1986. *Seashells of North America: A Guide to Field Identification*. Golden Book Publishing Company, New York.

Acosta, A. 1994. Bibliography of the conch genus *Strombus* (Gastropoda: Strombidae). *In:* R. S. Appeldoorn and B. Rodríguez, eds. *Proceedings of the Workshop on Biology, Fisheries, Mariculture and Management of the Queen Conch*, Strombus gigas. Fundación Científica Los Roques, Caracas, Venezuela. Pp. 321-356.

Aldana-Aranda, D., E. Baqueiro-Cárdenas, I. Martínez-Morales, R. I. Ochoa-Báez, and T. Brulé, 2003. Gonad behavior during peak reproduction period of *Strombus gigas* from Banco Chinchorro. *Bulletin of Marine Science* **73**: 241-248.

Alexander, C. G. and J. C. Rae. 1974. The structure and formation of the crystalline style of *Telescopium telescopium* (Linnaeus) (Gastropoda: Prosobranchia). *The Veliger* **17**: 56-60.

Bairati, A., M. Comazzi, and M. Gioria. 2001. An ultrastructural study of connective tissue in mollusc integument: II. Gastropoda. *Tissue & Cell* **33**: 426-438.

Brownell, W. N. and J. M. Stevely. 1981. The biology, fisheries, and management of the queen conch, *Strombus gigas*. *Marine Fisheries Review* **43**: 1-12.

Buckland, B. J. 1989. *Reproduction and Growth of the Queen Conch* Strombus gigas, *off St. Christopher and Nevis in the Eastern Caribbean*. M.Sc. Dissertation. University of Guelph, Ontario, Canada.

Checa, A. G. and A. P. Jiménez-Jiménez. 1998. Constructional morphology, origin, and evolution of the gastropod operculum. *Paleobiology* **24**: 109-132.

Creswell, L. 1994. An historical overview of queen conch mariculture. *In:* R. S. Appeldoorn and B. Rodríguez, eds. *Proceedings of the Workshop on Biology, Fisheries, Mariculture and Management of the Queen Conch*, Strombus gigas. Fundación Científica Los Roques, Caracas, Venezuela. Pp. 223-230.

Darcy, G. H. 1981. *Annotated Bibliography of the Conch Genus* Strombus *(Gastropoda, Strombidae) in the Western Atlantic Ocean*. NOAA Technical Report NMFS SSRF 748.

Dimitriadis, V. K. 2001. Structure and function of the digestive system in stylommatophora. *In:* G. M. Barker, ed., *The Biology of Terrestrial Molluscs*. CAB International, Landcare Research, Hamilton, New Zealand. Pp. 237-257.

Driscoll, A. L. 1972. Structure and function of the alimentary tract of *Batillaria zonalis* and *Cerithidea californica*, style-bearing mesogastropods. *The Veliger* **14**: 375-386.

Egan, B. D. 1985. *Aspects of the Reproductive Biology of* Strombus gigas. M.Sc. Dissertation, Department of Zoology. University of British Columbia, Vancouver, Canada.

Elston, R. A. 1990. *Mollusc Diseases: Guide for the Shellfish Farmer*. Washington Sea Grant Program, University of Washington Press, Seattle and London.

Fretter, V. and A. Graham. 1962. *British Prosobranch Molluscs*. Ray Society, London.

Gabe, M. 1968. *Techniques Histologiques*. Masson and Cie. Paris.

García-Cerruti, L. M. 1977. *Estudio del Comportamiento Fisiológico de la Hemocianina de* Strombus gigas. Tesis Profesional, Universidad Jorge Tadeo Lozano, Facultad de Ciencias del Mar. Bogotá, Colombia.

Gartner, L. P. and J. L. Hiatt. 1997. *Histología: Texto y Atlas*. McGraw Hill-Interamericana, México.

Greene, J. L. and A. J. Kohn. 1989. Functional morphology of the *Conus* proboscis (Mollusca: Gastropoda). *Journal of Zoology* **219**: 487-493.

Harris, J. O., C. M. Burke, and G. B. Maguire. 1998. Characterization of the digestive tract of the greenlip abalone, *Haliotis laevigata* Donovan. I. Morphology and histology. *Journal of Shellfish Research* **17**: 979-988.

Horiuchi, S. and C. E. Lane. 1965. Digestive enzymes of the crystalline style of *Strombus gigas* Linné. I. Cellullase and some other carbohydrases. *Biological Bulletin* **129**: 273-281.

Horiuchi, S. and C. E. Lane. 1966. Carbohydrases of the crystalline

style and hepatopancreas of *Strombus gigas* Linné. *Comparative Biochemistry and Physiology* **17**: 1189-1197.

Howard, D. W. and C. S. Smith. 1983. *Histological Techniques for Marine Bivalve Molluscs*. NOAA Technical Memorandum NMFSF/NEC-25. Woods Hole, Massachusetts.

Hughes, R. N. 1986. *A Functional Biology of Marine Gastropods*. Croom Helm Ltd., London and Sydney.

Jegla, T. C., and M. J. Greenberg. 1968. Structure of the bivalve rectum I. Morphology. *The Veliger* **10**: 253-263.

Kress, A., L. Schmekel, and J. A. Nott. 1994. Ultrastructure of the digestive gland in the opisthobranch mollusk, *Runcina*. *The Veliger* **37**: 358-373.

Little, C. 1965. Notes on the anatomy of the queen conch *Strombus gigas*. *Bulletin of Marine Science* **15**: 338-358.

Little, C. 1967. Ionic regulation in the queen conch, *Strombus gigas* (Gastropoda, Prosobranchia). *Journal of Experimental Biology* **46**: 459-474.

Morton, J. E. 1979. *Molluscs*, 5th Ed. Hutchinson, London.

Owen, G. 1966. Digestion. *In:* K. M. Wilbur and C. M. Yonge, eds., *Physiology of Mollusca*. Vol 2. Academic Press, New York. Pp. 53-96.

Randall, J. E. 1964. Contributions to the biology of the queen conch, *Strombus gigas*. *Bulletin of Marine Science of the Gulf and Caribbean* **14**: 246-295.

Ray-Culp, M. 2003. The Conch News website: Welcome to conch news. Available at: http://bellsouthpwp.net/c/u/culpsb/conchnews/welcome.html 31 December 2003

Reed, S. E. 1993. Gonadal comparison of masculinized females and androgynous males to normal males and females in *Strombus* (Mesogastropoda: Strombidae). *Journal of Shellfish Research* **12**: 71-75.

Reed, S. E. 1995a. Reproductive anatomy and biology of the genus *Strombus* in the Caribbean: I. Males. *Journal of Shellfish Research* **14**: 325-330.

Reed, S. E. 1995b. Reproductive anatomy and biology of the genus *Strombus* in the Caribbean: II. Females. *Journal of Shellfish Research* **14**: 331-336.

Salvini-Plawen, L. v. 1988. The structure and function of molluscan digestive systems. *In:* E. R. Trueman and M. R. Clarke, eds., *The Mollusca, Form and Function*, Vol 11. Academic Press, Inc., London. Pp. 301-379.

Sminia, T., W. P. W. van der Knaap, and L. A. Van Asselt. 1983. Blood cell types and blood cell formation in gastropod molluscs. *Developmental and Comparative Immunology* **7**: 665-668.

Stoner, A.W. 1997. The status of queen conch, *Strombus gigas*, research in the Caribbean. *Marine Fisheries Review* **59**: 14-22.

Stoner, A. W. and J. M. Waite. 1991. Trophic biology of *Strombus gigas* in nursery habitats: Diets and food sources in seagrass meadows. *Journal of Molluscan Studies* **57**: 451- 60.

Thompson, J. T., A. D. Lowe, and W. M. Kier. 1998. The columellar muscle of prosobranch gastropods: Morphological zonation and its functional implications. *Invertebrate Biology* **117**: 45-56.

van der Knaap, W. P. W., C. M. Adema, and T. Sminia. 1993. Invertebrate blood cells: Morphological and functional aspects of the haemocytes in the pond snail *Lymnaea stagnalis*. *Comparative Haematology International* **3**: 20-26.

Voltzow, J. 1990. The functional morphology of the pedal musculature of the marine gastropods *Busycon contrarium* and *Haliotis kamtschatkana*. *The Veliger* **33**: 1-19

Voltzow, J. 1994. Gastropoda: Prosobranchia. *In:* F. W. Harrison and A. J. Kohn, eds., *Microscopic Anatomy of Invertebrates*, Vol. 5, Mollusca I. John Wiley and Sons Ltd., New York. Pp. 111-252.

Ward, J. 1966. Feeding, digestion, and histology of the digestive tract in the keyhole limpet *Fissurella barbadensis* Gmelin. *Bulletin of Marine Science* **16**: 668-684.

Warren, A. 1959. *Textbook of Comparative Histology*. Oxford University Press, New York.

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Daedalochila sp. nov. from northwest Arkansas, U.S.A., the anatomy of the *Polygyra plicata* group, and the validity of the genus *Millerelix* Pratt, 1981 (Gastropoda: Pulmonata: Polygyridae)

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Abstract: The species of the *Polygyra plicata* group Pilsbry, 1940 of the Polygyridae, Polygyrini - *Polygyra plicata* Say, 1821; *Polygyra dorfeuilliana* Lea, 1838; *Polygyra fatigiata* Say, 1829; *Polygyra jacksoni* (Bland, 1866); *Polygyra peregrina* Rehder, 1932; and *Polygyra troostiana* Lea, 1839 - have been placed in the genus *Millerelix* Pratt, 1981 on the basis of limited anatomical data. We present the genital anatomy of these species and describe a new species from the Ozark uplift of Arkansas, U.S.A., *Daedalochila bisontes* sp. nov. The new species is similar to *P. peregrina*. It is distinguished by its smaller size, more closely coiled and weakly striate shell, and by the structure of the lamellae of the apertural lip. The parietal lamella bears a prominent angled projection apically and an obtuse angle basally. The palatal lamella is deeply immersed and long, curving inwards and downwards towards the basal lamella, sinuous and undulate, appearing in apertural view as two overlapping lamellae. The diagnostic character of *Millerelix* was not consistently present in the *Polygyra plicata* group; *i.e.*, the pendant, conical projection in the apical penis was observed only for *P. plicata* itself and not confirmed for *P. dorfeuilliana*. The diagnostic characters of *Millerelix* (*Prattelix*) Emberton, 1995 and *Millerelix* (*Millerelix*) were also found to be unreliable; *i.e.*, a thickened proximal vas deferens was found in all 6 species and the width/length of the penis (0.10-0.18) was similar in *Polygyra* (*Millerelix* [*Millerelix*]) *dorfeuilliana* and *Polygyra* (*Millerelix* [*Prattelix*]) *plicata*. *P. troostiana* and *P. fatigiata* possess penises of greater width/length (~0.36) and lack any trace of an epiphallus, features that appear to place them (anatomically) close to *Daedalochila* (*Upsilidon*) Pilsbry, 1940. Because of the variability of these features, *Millerelix* Pratt, 1981 is not maintained, and the species of the *Polygyra plicata* group are referred to the senior genus *Daedalochila* Beck, 1837.

Key words: endemism, genital morphology, Ozark uplift, taxonomy

The *Polygyra plicata* group was established by Pilsbry (1940) for the Polygyrinae of the Cumberland plateau and Ozark uplift of the southern U.S.A., i.e., *Polygyra plicata* Say, 1821; *Polygyra dorfeuilliana* Lea, 1838; *Polygyra fatigiata* Say, 1829; *Polygyra jacksoni* (Bland, 1866); *Polygyra peregrina* Rehder, 1932; and *Polygyra troostiana* Lea, 1839. Pilsbry (1940) gave no formal rank to the group, placing the species in the (then) subgenus *Daedalochila* Beck, 1837. The group remained without a formal name until the most recent revision of the Polygyridae by Emberton (1995), who extended the concept of the genus *Millerelix* Pratt, 1981 (originally erected for several Texan species of Polygyrini [Pratt 1981a, 1981b]) to include all members of the *Polygyra plicata* group. *Millerelix* has been used in this sense by subsequent workers (Turgeon *et al.* 1998).

During field work on the distribution of land snails in Arkansas, U.S.A. (Coles and Walsh 1999, Walsh and Coles 2002), a polygyrid snail of the *Polygyra plicata* group was found that was distinct from other related species, although superficially similar to *Polygyra peregrina* of the same region. When the anatomy of the new form was studied, it became apparent that it did not conform to the diagnostic genital characters of the genus *Millerelix*, either as defined by Emberton (1995) to include the entire *Polygyra plicata* group or as originally described by Pratt (1981a, 1981b).

To gain more insight into the validity of the concept of a single genus to describe the members of the *Polygyra plicata* group, we describe the new Arkansas species and present dissections of all the species within the group. In particular, we present illustrations of the internal features of the penis because these have been used by both Pratt (1981a, 1981b) and Emberton (1995) as diagnostic generic characters (Table 1) but have not been illustrated, even for those few species for which the anatomy is known.

MATERIALS AND METHODS

Specimens of Polygyridae were collected as part of our studies on the distribution of land molluscs of Arkansas, Tennessee, and Alabama. The identities of the species were ascertained by reference to Pilsbry (1940) and the collections of the Field Museum of Natural History, Chicago (FMNH). Additional specimens of the new species were located in the Hubricht collection at the FMNH and the Causey collection at the University of Arkansas Museum, Fayetteville (UAF). Live specimens of all members of the *Polygyra plicata* group

Table 1. Characters used to define genera and subgenera within the *Millerelix/Daedalochila* clade and the species assigned to the genera/subgenera by Emberton (1995).

Genus (Subgenus)	Characters (character set of Emberton 1995: 77)	Species[1]
Millerelix	A slender penis (width ≤0.12 length) with an apical, pendant, conical projection, and derivatives thereof (87).	
(*Millerelix*)	An extremely long and slender penis (width <0.06 length) (88).	***mooreana***, *dorfeuilliana, gracilis*[2], *?implicata, lithica, ?rhoadsi, tholus.*
(*Prattelix*)	A greatly enlarged, muscular, proximal vas deferens (89).	***plicata***, *deltoidea*[3], *fatigiata, jacksoni, peregrina, plicata, simpsoni*[3], *troostiana.*
Daedalochila	Even-diameter vas deferens with no trace of epiphallus (90).	
(*Upsilidon*)	A stout penis (length/diameter <3.5 [diameter/length >0.29]) with a straight apex (91).	***hippocrepis***, *?acutedentata, burlesoni, chisosensis, dalli, leporina, multiplicata, ?poeyi, sterni.*
(*Daedalochila*)	A moderately long penis (4< length/diameter <10 [0.1< diameter/length <0.25]) with a bent or convoluted apex (92). A downward curve on the lower limb of the parietal apertural denticle (93). A raised parietal callus (94).	***auriculata***, *?ariadne, auriformis, avara, delecta, hausmani, ?oppiliata, peninsulae, postelliana, subclausa, uvulifera.*

[1] The type species is given first, in bold (for author citations see text and below).

[2] Regarded as a form of *mooreana* by some workers (*cf.* Pratt 1981b). ? indicates species provisionally assigned.

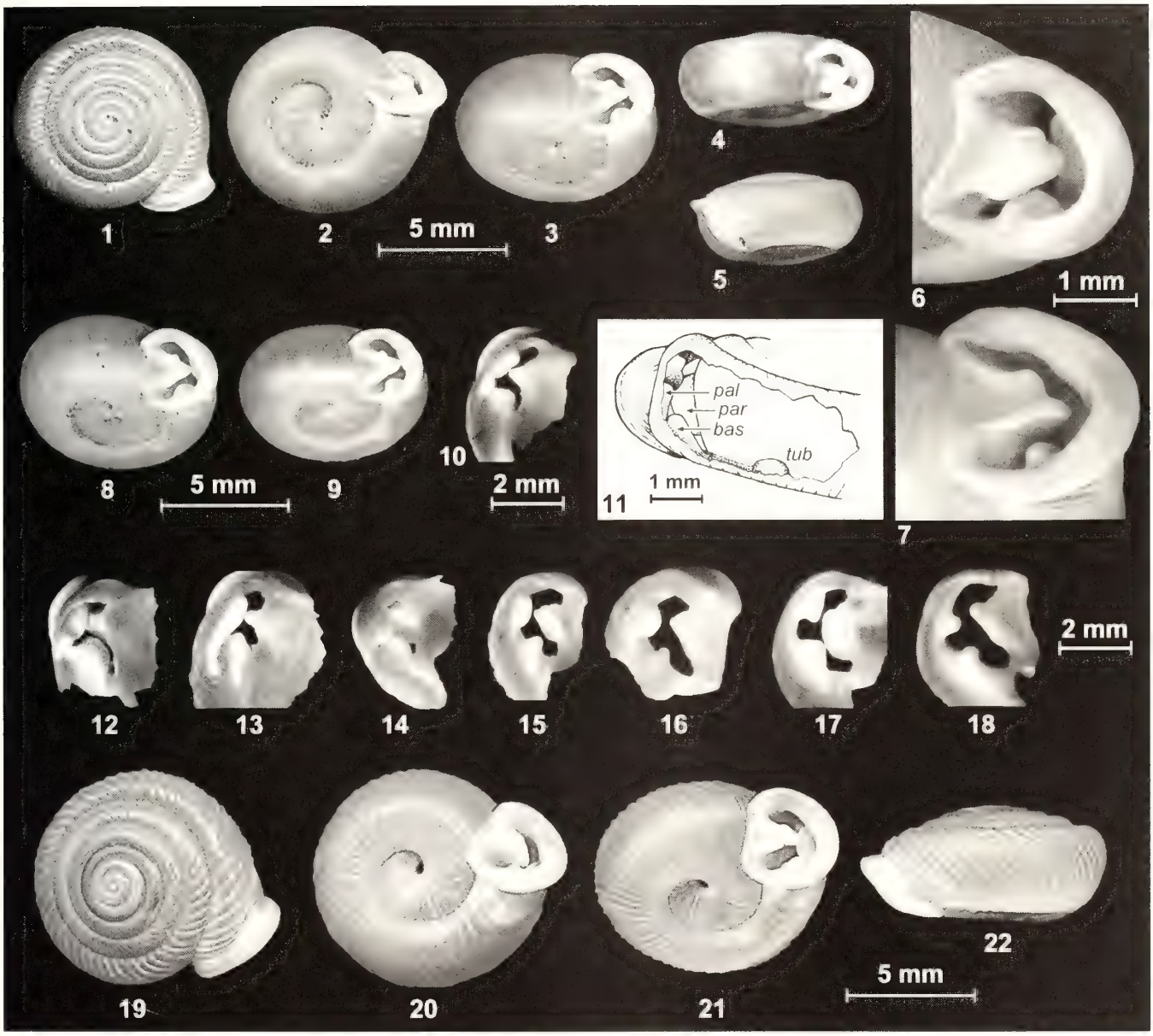
[3] Regarded as forms of *jacksoni* by Pilsbry (1940).

Author citations for species not otherwise referred to in the text are as follows. *Millerelix (Millerelix) implicata* (Martens, 1865); *Millerelix (Millerelix) rhoadsi* (Pilsbry, 1900); *Millerelix (Millerelix) tholus* (Binney, 1857); *Millerelix (Prattelix) deltoidea* (Simpson, 1889); *Millerelix (Prattelix) simpsoni* (Pilsbry and Ferriss, 1907). *Daedalochila (Upsilison) hippocrepis* (Pfeiffer, 1848); *Daedalochila (Upsilidon) acutedentata* (Binney, 1858); *Daedalochila (Upsilidon) burlesoni* (Metcalf and Riskind, 1979); *Daedalochila (Upsilidon) chisosensis* (Pilsbry, 1936); *Daedalochila (Upsilidon) dalli* (Metcalf and Riskind, 1979); *Daedalochila (Upsilidon) leporina* (Gould, 1948); *Daedalochila (Upsilidon) multiplicata* (Metcalf and Riskind, 1979); *Daedalochila (Upsilidon) poeyi* (Aguayo and Jaume, 1947); *Daedalochila (Upsilidon) sterni* (Metcalf and Riskind, 1979); *Daedalochila (Daedalochila) auriculata* (Say, 1818); *Daedalochila (Daedalochila) ariadne* (Pfeiffer, 1848); *Daedalochila (Daedalochila) auriformis* (Bland, 1862); *Daedalochila (Daedalochila) avara* (Say, 1818); *Daedalochila (Daedalochila) delecta* (Hubricht, 1976); *Daedalochila (Daedalochila) hausmani* (Jackson, 1948); *Daedalochila (Daedalochila) oppiliata* (Morelet, 1849); *Daedalochila (Daedalochila) peninsulae* (Pilsbry, 1940); *Daedalochila (Daedalochila) postelliana* (Bland, 1862); *Daedalochila (Daedalochila) subclausa* (Pilsbry, 1899); *Daedalochila (Daedalochila) uvulifera* (Shuttleworth, 1852).

were obtained during these studies. Specimens were drowned overnight in sealed containers of degassed (boiled and cooled) water and preserved in 70% ethanol.

Images of the entire shell were obtained using a Hewlett Packard Scanjet 4C at a resolution of 600 dpi (Figs. 1-5, 8-9, 19-22). Shells were laid directly on the scanner platten or fixed at a desired angle using a probe. Images (as TIFF files) were adjusted empirically for contrast and brightness (and also to remove the image of the probe) using Adobe Photoshop®. Details of the aperture (Figs. 6-7) were obtained by scanning electron microscopy (SEM), using a Hitachi S-2460N, with AC voltage at 22 kV in N-SEM mode at 10 Pa vacuum. The shell was not coated with heavy metal. For details of these specimens see legend to Figs. 1-22.

Views of the apertural lamellae of the shell from behind the aperture (Figs. 10, 12-18) were obtained by removing pieces of the shell from behind the aperture until a clear view of the lamellae was possible. The resultant apertural fragments were placed aperture-down on the base of a Zeiss SM2U dissecting microscope attached to a MTI DCC30 digitizing camera. Lighting was adjusted empirically until the best views of the lamellae were obtained. Images were acquired using FlashPoint and adjusted for contrast and brightness using Adobe Photoshop®. The specimens were: *Daedalochila bisontes* sp. nov. (Fig. 10), type locality, B. Coles, 8 May 1999 (FMNH293229); *Daedalochila jacksoni* (Fig. 12), Arkansas, Franklin County, Reed Mountain Park, Ozark, B. Coles, 18 January 1999 (FMNH293235); *Daedalochila peregrina* (Fig. 13), Arkansas, Searcy County, Highway 65 at junction with highway 74, 5 miles N of Marshall, B. Coles, 3 May 1998 (FMNH293231); *Daedalochila plicata* (Fig. 14), Tennessee, Van Buren County, Bone Cave Mountain 5 miles NE of Spencer, B. Coles, 20 June 1996 (FMNH293237); *Daedalochila troostiana* (Fig. 15), Tennessee, De Kalb County, Cove Hollow Road at N end of Center Hill Lake, B. Coles, 16 April 1999 (FMNH293243); *Daedalochila fatigiata internuntia* (Fig. 16), Tennessee, Perry County, Perryville, Mousetail Landing State Park, B. Coles, 23 March 2000 (FMNH293244); *Daedalochila dorfeuilliana* (Fig. 17), Arkansas, Hempstead county, Saratoga Landing, B.



Figures 1-22. Shell characters of *Daedalochila bisontes* sp. nov. and related species. Figs. 1-5, *D. bisontes*, flat-bed scans of the holotype (FMNH287396). Figs. 6-7, *D. bisontes*, SEMs of the aperture of the holotype. Figs. 8-9, *D. bisontes*, flat-bed scans of two paratypes (FMNH293226). Fig. 10, *D. bisontes*, apertural fragment showing detail of lamellae viewed from behind the aperture (FMNH293229). Fig. 11. *D. bisontes*, sketch of shell opened behind aperture to show the appearance of the palatal lamella (*pal*) as two overlapping lamellae and the tubercle (*tub*) on the umbilical axis. Figs. 12-18, apertural fragments of shells of the *Polygyra plicata* group and related species viewed from behind the aperture: Fig. 12, *Daedalochila jacksoni*; Fig. 13, *Daedalochila peregrina*; Fig. 14, *Daedalochila plicata*; Fig. 15, *Daedalochila troostiana*; Fig. 16, *Daedalochila fatigiata internuntia*; Fig. 17, *Daedalochila dorfeuilliana*; Fig. 18, *Daedalochila mooreana.* Figs. 19-22, *Daedalochila peregrina*, flat-bed scans (Arkansas, Calico Rock, Stone County [FMNH293232]). Views of entire shells are at the same scale (scale bar = 5 mm). For views of apertural fragments, scale bar = 2mm; for SEMs scale bar = 1 mm. For apertural fragments, the orientation of the lamellae is shown on Fig. 11, otherwise, shells are viewed in the conventional way with the parietal lamella to the left and palatal lamella to the right. Abbreviations: *bas*, basal lamella; *pal*, palatal lamella; *par*, parietal lamella.

Coles, 5 January 2003 (FMNH293239); *Daedalochila mooreana* (Fig. 18), Texas, Comal County, bluffs of Guadeloupe River, approximately 6 miles W of New Braunfels, B. Coles, 24 December 1997 (FMNH293246).

Shell measurements were taken using vernier calipers. Shell diameter and height were measured as described by Pilsbry (1939). Measurements were taken at least twice and the average used. Replicate measurements were within ± 0.05 mm. Whorls of the spire were counted (at 4X magnification) as described by Pilsbry (1939) and umbilical whorls counted similarly, using the central umbilical hole as the starting point; accuracy was to ± 0.05 whorl. Measurements were taken of 126 specimens of *Daedalochila bisontes,* including the holotype, unbroken paratypes, and other material marked with an asterisk (*) in *Paratypes* and *Other Material* and 144 specimens of *Daedalochila peregrina*; that is, 58 shells from Calico Rock, Stone County, B. Coles, 16 April 1995 (FMNH293233); 49 shells from Allison, Stone County, B. Coles, 28 February 1999 (FMNH293234), and 37 shells from Searcy County, Highway 65 at junction with Highway 74, 5 miles N of Marshall, B. Coles, 21 April 2002 (FMNH293232). These data were analyzed by box plots, simple graphical plots, and by comparison of means for significant differences by Student's t-test using the programs SigmaStat and SigmaPlot.

Dissections were performed at 20-40X magnification. The shell was removed from the body, the animal opened from near the genital pore to the lung, and the tissues teased apart while submerged in 70% ethanol. The right ocular retractor muscle was cut and partially removed to aid viewing the basal penis. The penis was opened with a sharpened spear-point needle from near its junction with the atrium/vagina to the epiphallus or to the attachment of the penial retractor muscle (or as far as the fragility of the tissues would allow). Sections of the penis were obtained by cutting the opened penial tubes where indicated in the figures and allowing the remaining tissues to reform to their approximate original cylindrical forms. Drawings were made by eye, taking particular care to ensure that relative proportions of the organs were accurately reproduced, and with reference to an appropriate scale.

The nomenclature used for the apertural lamellae (equivalent to the apertural "teeth" or "denticles" of other authors) and the concordance with the nomenclature of Pilsbry (1940) in parentheses, is as follows: parietal lamella (parietal tooth), palatal lamella (outer lip tooth), basal lamella (basal lip tooth). Abbreviations are used as follows: Buffalo National River (BNR), Carnegie Museum of Natural History (CM), Field Museum of Natural History (FMNH), Florida Museum of Natural History (UF), University of Arkansas Museum Fayetteville (UAF).

SYSTEMATICS

Class GASTROPODA
Subclass PULMONATA
Order STYLOMMATOPHORA
Family POLYGYRIDAE Pilsbry, 1894
Subfamily POLYGYRINAE
Tribe POLYGYRINI
Genus *Daedalochila* Beck, 1837 [*non Daedalochila sensu* Emberton, 1995]
Polygyra plicata group Pilsbry, 1940

Daedalochila bisontes sp. nov.
[undescribed *Millerelix* sp. nov. Coles and Walsh 1999, Walsh and Coles 2002]
Figs. 1-11, 23-25, 40-41

Diagnosis

A medium-sized species of the *Polygyra plicata* group, similar in aspect to *Daedalochila peregrina*; depressed-discoidal, weakly rib-striate above, umbilicus perforate expanding to approximately 2/3 diameter of shell, forming an almost planar spiral; shell aperture with three lamellae; parietal lamella triangular-quadrate, a prominent angled projection apically and obtuse angle basally; palatal lamella deeply immersed and long, curving inwards and downwards towards the basal lamella, sinuous and undulate, appearing in apertural view as two overlapping lamellae; a tubercle on the umbilical axis approximately 1/4 whorl inside body whorl.

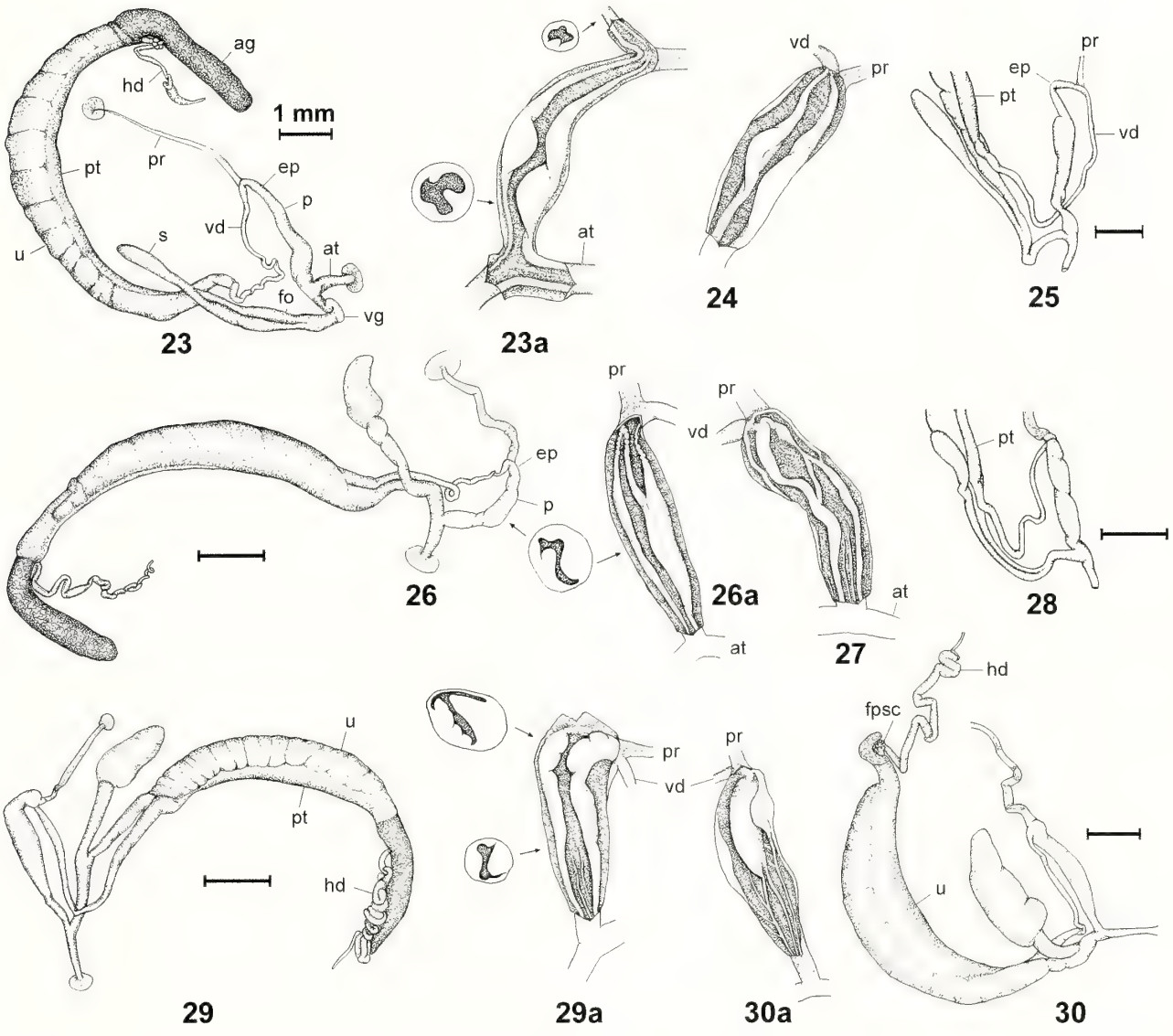
Holotype (Figs. 1-7)

FMNH287396, preserved dry: U.S.A., Arkansas, Searcy County; Leslie, approximately 1 mile S of town center, just within city limits, opposite stone quarry yard, a roadside bank with limestone outcrops on W side of Highway 65, on soil under kudzu and other herb cover; B. Coles, 13 May 1998.

Paratypes (* indicates measured).

Arkansas, Madison County: UAF95-1-330 (Causey Collection), 5 specimens preserved dry: Denny's Cave, Huntsville, Etgay (no initials given), May 1954.

Arkansas, Searcy County: *FMNH287397, 29 specimens preserved dry: collected with holotype. *FMNH293226, 2 specimens preserved dry (Figs. 8-9): collected with holotype. FMNH287398, 6 specimens preserved in alcohol: collected with holotype. FMNH287399, 4 specimens dissected and preserved in alcohol: collected with holotype. FMNH293227, 4 specimens dissected, preserved in alcohol: type locality, B. Coles, 8 May 1999. FMNH293229, 1 apertural fragment (Fig. 10): type locality, B. Coles, 8 May 1999. FMNH255872, 5 specimens preserved dry: 2 miles SE of Marshall, L. Hu-



Figures 23-30. Genital anatomy of *Daedalochila bisontes, Daedalochila peregrina,* and *Daedalochila jacksoni.* Fig. 23, *Daedalochila bisontes* sp. nov., genitalia of a specimen with weakly differentiated epiphallus; Fig. 23a, the penis of the same specimen opened from the vagina/atrium to the vas deferens, insets show sections of the penis and vas deferens. Fig. 24, *D. bisontes,* opened penis of a second specimen. Fig. 25, *D. bisontes,* distal genitalia of a third specimen showing well-defined and bent epiphallus. Fig. 26, *Daedalochila peregrina,* genital anatomy; Fig. 26a, opened penis and penial section (inset) of the same specimen. Fig. 27, *D. peregrina,* opened penis of a second specimen. Fig. 28, *D. peregrina,* distal genitalia of a third specimen. Fig. 29, *Daedalochila jacksoni,* genital anatomy; Fig. 29a, opened penis and penial sections (insets) of the same specimen. Fig. 30, *D. jacksoni,* distal genital anatomy of a second specimen; Fig. 30a, opened penis of the second specimen showing pilasters weakly developed basally. Scale bars are all 1 mm and refer to the gross genitalia only. Abbreviations: ag, albumin gland; at, atrium; ep, epihallus; fo, free oviduct; fpsc, fertilization pouch-seminal receptacle complex; hd, hermaphrodite duct; p, penis; pr, penial retractor muscle; pt, prostate gland; s, spermatheca; u, uterus; vd, vas deferens; vg, vagina.

bricht, 31 July 1955. FMNH293228, 1 specimen dissected, plus 1 entire, preserved in alcohol: Leslie, Kiwanis Road, B. Coles, 21 April 2002. *UF278302, 8 specimens preserved dry: type locality: J. Slapcinsky and B. Coles, 8 May 1999. UF278303, 4 specimens preserved in alcohol: type locality, J. Slapcinsky and B. Coles, 8 May 1999. *UF278317, 23 specimens preserved dry: Leslie, County Road 9 (Kiwanis road), J. Slapcinsky and B. Coles, 8 May 1999. *CM65377, 10 specimens preserved dry: Leslie, type locality, B. Coles, 13 May 1998.

Arkansas, Newton County: FMNH255871, 3 specimens preserved dry: 12 miles S of Jasper, L. Hubricht, 29 April 1936.

Arkansas, Stone County: FMNH287400, 3 specimens preserved dry: 5 miles N of Allison, L. Hubricht, 9 February 1960.

Other material (* indicates measured).

Arkansas, Newton County: Two specimens: Carver, Gene Rush-Buffalo River Wildlife Management Area, 1 mile E of Highway 123, B. Coles, 1 May 1998. 17 Specimens: Center Point Trail, Buffalo National River (BNR), G. Walsh, 23 May 1997. *4 specimens: Lost Valley Trail BNR, G. Walsh, 23 September1996. *10 Specimens: Hemmed in Hollow, BNR, G. Walsh, 17 June 1997.

Arkansas, Searcy County: *25 Specimens: Leslie type locality, B. Coles, 8 May 1999. 19 Specimens: Leslie, Kiwanis Road, B. Coles, 7 May 1998 and 21 April 2002. 3 specimens: Marshall, Highway 65, 2 miles SE of town, B. Coles, 16 May 1998 and 8 May 1999. 10 specimens: Tyler Bend, River View Trail, BNR, G. Walsh, 30 September 1995. 3 specimens: Tyler Bend, Spring Hollow Trail, BNR, G. Walsh, 30 September 1995. *18 specimens: Tyler Bend, Center Point, Buffalo River Hiking Trail BNR, G. Walsh, 10 January 1995.

Description

Shell (Figs. 1-9) mid - light brown, discoidal, diameter 6.15-7.96 mm (mean 7.14 mm, holotype 7.35 mm); height 2.50-3.31 mm (mean 2.89 mm, holotype 2.67 mm). Closely wound; whorls 5.25-6.20 (mean 5.73, holotype 5.80); spire low; periphery rounded; umbilicus perforate, expanding to form an open almost planar spiral; umbilical whorls 1.00-1.60 (mean 1.25, holotype 1.25), umbilicus (between sutures) approximately 1/2 maximum shell diameter. Embryonic sculpture of weak axial striae, subsequently weakly rib striate, the rib-striae becoming obsolete as they cross the periphery, but stronger behind the aperture. Shell aperture rounded; peristome strongly thickened, reflected, ends connected by a low parietal callus appressed to the body whorl, porcellaneous, appearing slightly rough at 40X magnification (Figs. 6-7). Aperture bears parietal, basal, and palatal lamellae. Parietal lamella triangular-quadrate in apertural view, a prominent projection approximately midway on apical surface, an obtuse angle basally. The basal lamella emerges onto the lower peristome and extends approximately 0.1 whorls into the body whorl. The palatal lamella is deeply immersed and long, curving inwards and downwards towards the basal lamella, sinuous and undulate (Figs. 10-11), appearing in apertural view as two overlapping lamellae. Internally, there is a tubercle on the umbilical axis approximately 1/4 whorl inside the body whorl (Fig. 11). Unworn (young adult) shells possess sparse short (approximately 0.5 mm long) periostracal hairs primarily on the basal surface.

Genital anatomy

Nine specimens were dissected from the type locality (FMNH 287399, FMNH293227, FMNH293228); the penis opened in two specimens (Figs. 23-25). Atrium approximately 1/4 the length of the penis, vagina approximately twice as long as the atrium, strongly reflexed mid-way. Free oviduct to the bend in the vagina approximately the same length as the penis, 2-3 mm long. Penis elongate, approximately 2.5 mm in length variably swollen at mid-length, maximum width/length 0.10-0.13 (mean = 0.12, n = 6) including the epiphallus, which varies from being straight and weakly-defined (Fig. 23) to distinct and bent (Fig. 25); without appendix or flagellum; penial retractor muscle terminal. Vas deferens narrow, expanding proximally from approximately mid-length to reach its maximum diameter (about 2-3 times its minimum diameter) at the junction with the prostate gland. Internally, the penis bears two fleshy pilasters developed into lobes or lamellae in the mid-penis (Figs. 23a, 24). In the terminal penis the pilasters are weakly developed, thus defining the epiphallus, and extend into the vas deferens as weakly-defined ridges of the thickened epiphallar wall (Fig. 23a); towards the base of the penis the pilasters are low ridges that extend into the atrium and vagina (Fig. 23a). No papillae, glandulose regions, colored regions, or other additional features were visible at the magnification used (40X).

Comparison with related species

Daedalochila bisontes conforms to the *Polygyra plicata* group of Polygyridae on the basis of its depressed, openly umbilicate shell; triangular-quadrate parietal tooth with a callus appressed to the body whorl; the presence of basal and palatal lamellae; and its geographical localization (Pilsbry 1940: 625).

In general aspect, *Daedalochila bisontes* most closely resembles *Daedalochila peregrina* (Figs. 19-22); that is, the rounded periphery, open umbilicus, and deeply placed palatal lamella. On casual inspection the parietal lamella of *D. bisontes* resembles that of *D. peregrina*, but the prominent projection on the apical side and the angle basally (Figs. 3-4, 6-9) are constant features of all known adult specimens of *D. bisontes* and are not features that develop only in gerontic individuals of the species. Figs. 8 and 9 represent the range of variation of shape of the parietal lamella in the paratype series. The parietal lamella of *D. peregrina* has only an irregularity on the apical side, where it bends into the aperture; the basal side is straight or slightly curved (Figs. 20-21 and see Pilsbry 1940, Fig. 397:9). The two species are distinguished further by the generally smaller size of *D. bisontes*, the more closely coiled whorls, and the greater number of umbilical whorls (Figs. 40-41). In contrast to *D. peregrina, D. bisontes* shows weak rib striation that is present on the apical surface only (and behind the aperture) (Figs. 1-5). Although striation is regarded as a variable feature (for example, in *Daedalochila fatigiata* [Pilsbry 1940: 628-629] and *Daedalochila dorfeuilliana* [Pilsbry 1940: 634-637, 633, fig. 398]), this

is a constant difference between all known specimens of *D. bisontes* and all the *D. peregrina* that we have examined.

In the form of the parietal lamella *Daedalochila bisontes* bears some resemblance to *Daedalochila plicata* (Pilsbry 1940, Fig. 397:1), *Daedalochila fatigiata* (Pilsbry 1940, Fig. 397:4) and *Daedalochila troostiana* (Pilsbry 1940, Fig. 397:8), which also possess a projection on the apical side of the parietal lamella and an obtuse angle on the basal side. However, the deeply immersed and long palatal lamella curving inwards and downwards into the body whorl towards the basal lamella distinguishes *D. bisontes* from *D. troostiana, D. fatigiata, D. dorfeuilliana, Daedalochila lithica* (Hubricht, 1961) and *Daedalochila mooreana* (W.G. Binney, 1857) (also from *Linisa texasiana* [Moricand, 1833] and related species). This feature is most clearly seen by viewing the lamellae from "behind" the aperture after removal of the body whorl. Figs. 10-18 illustrate such views for all the well-defined species of the *Polygyra plicata* group and for *Daedalochila mooreana,* the type species of *Millerelix.* The palatal lamella is usually visible externally as a whitish line that forms an arc that defines a smooth, raised area behind the peristome. For examples, see Pilsbry 1940: pp. 632 & 627, Fig. 397:14 (*Daedalochila jacksoni*); pp. 626-627, Fig. 397:1 (*D. plicata*); p. 631 and Fig. 22 of this report (*D. peregrina*); and Fig. 5 of this report (*D. bisontes*). *D. plicata* lacks an internal tubercle on the umbilical suture, the basal lamella extends into the body whorl to reach the umbilical suture (Fig. 14) (see also Pilsbry 1940: 626-627), and is also distinct in internal morphology of the penis (see below). *D. jacksoni* has a palatal lamella very similar to that of *D. bisontes* (compare Figs. 10 and 12), but in general form, the shell of *D. jacksoni* presents a very different aspect, being more inflated with a rounded base, and possessing a higher spire and a short umbilical suture (Pilsbry 1940, Fig. 397:11-15). *D. jacksoni* is further distinguished by the strong tongue-like parietal lamella, the lack of an internal tubercle, and the morphology of the penis (see below).

An apparently unique feature of *Daedalochila bisontes* is the appearance of the palatal lamella as two overlapping lamellae when viewed through the aperture. This arises because the tooth is sinuous and undulate, being high at its outer and inner ends, with a low mid-portion so that only the two higher portions are visible through the aperture. This feature is easily seen with shells "in the hand" where light and angle can be readily manipulated, but is not well defined in Figures 3, 4, and 6-9 because of difficulties of lighting and limitation of depth of field. Figure 11 shows this aspect viewed from behind the aperture. This feature is absent in *Daedalochila peregrina*, which possesses a palatal lamella that becomes more uniformly low toward the body whorl (Fig. 13), thus further distinguishing *D. peregrina* and *D. bisontes*. In *Daedalochila jacksoni* the inner end of the palatal lamella (viewed through the aperture) is obscured by the strong parietal lamella (see Pilsbry, 1940, Fig. 397:11-14).

In practice, specimens of *Daedalochila bisontes* can be identified without recourse to opening the shell by the combination of rounded periphery, open and almost planar umbilical spiral, the weak striation that is obsolete below the periphery, and the projections on the parietal lamella. Identity can be confirmed by examining the structure of the palatal lamella as viewed through the aperture and behind the peristome.

Distribution

The localities to date are in a restricted area close to the Buffalo River region of the Ozark Mountains of Arkansas, U.S.A. All sites are on limestone outcrops. Thus, *Daedalochila bisontes* appears to represent an Arkansas-Ozarkian endemic species. Several other species of Polygyridae that are either endemic to Arkansas or are of restricted distribution are also known from this area (Hubricht 1985, Coles and Walsh 1999, Walsh and Coles 2002), namely *Daedalochila peregrina*, *Patera clenchi* (Rehder, 1932), and *Triodopsis neglecta* (Pilsbry, 1899). At the type locality, *D. bisontes* was found sympatric with *Daedalochila dorfeuilliana*. A single lot (FMNH267965: Arkansas, Stone County, White River, 0.5 miles N of Allison, L. Hubricht, 2 September 1960) contained *D. peregrina*, *Daedalochila jacksoni*, and *D. bisontes* (these last recataloged as FMNH287400). We have seen only *D. peregrina* and *D. dorfeuilliana* from this area and Hubricht (1985) does not report Stone County, Arkansas, for *D. jacksoni. D. bisontes* appears to have been overlooked because of its superficial similarity to small specimens of *D. peregrina*.

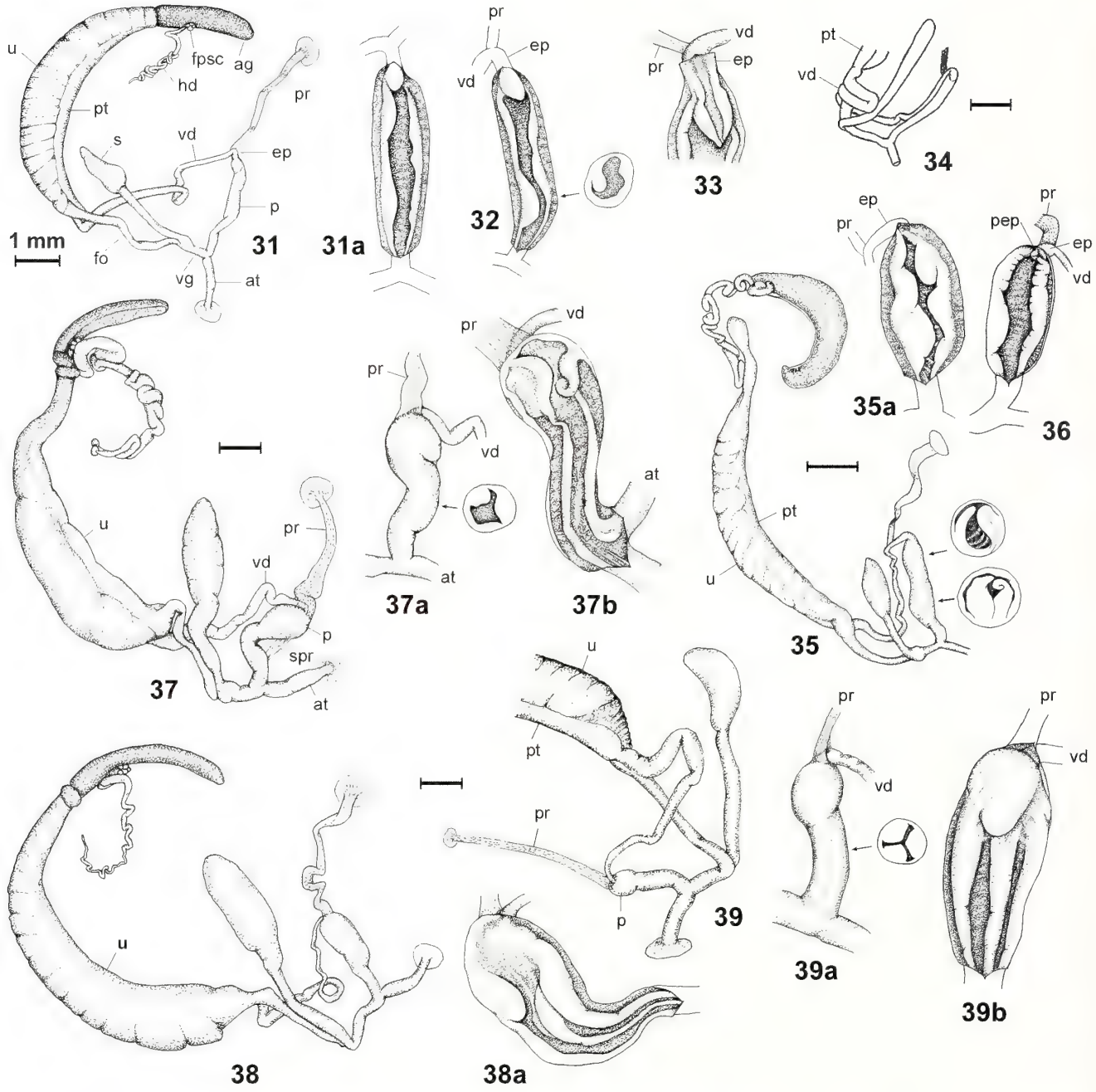
Etymology

The specific epithet *bisontes* is derived from the early modern English word for bison and refers to the known localities in the region of the Buffalo National River of Arkansas. The vernacular name *Buffalo River liptooth* is proposed for the species.

GENITAL ANATOMY OF THE *POLYGYRA PLICATA* GROUP

General features

The genitalia of all specimens dissected were of the general form described for *Daedalochila bisontes.* That is, in the approximate relative proportions of the atrium, vagina, penis, and free oviduct; the attachment of the penial retractor muscle at the apex of the penis; the lack of flagellum or appendix; and the thickening of the proximal vas deferens at its junction with the prostate gland. Differences between species, particularly with respect to the shape of the penis, its



Figures 31-39. Genital anatomy of *Daedalochila plicata, Daedalochila dorfeuilliana, Daedalochila troostiana,* and *Daedalochila fatigiata.* Fig. 31, *Daedalochila plicata*, genital anatomy; Fig. 31a, opened penis of same specimen. Fig. 32, *D. plicata,* opened penis of a second specimen and section of the penis (inset). Fig. 33, *D. plicata*, details of the apical pendant conical projection of the penis in a third specimen (viewed from opposite side of penis compared to Figs. 31a, 32). Fig. 34, *D. plicata*, distal genitalia of a fourth specimen in a less disturbed state. Fig. 35, *Daedalochila dorfeuilliana,* genital anatomy and sections of the penis (insets); Fig. 35a, penis of the same specimen opened from near the vagina/atrium to the epiphallus. Fig. 36, *D. dorfeuilliana*, penis of a second specimen showing the junction of the penis and epiphallus, puckering of the pilasters, and puckering of the epiphallar walls (pep). Fig. 37, *Daedalochila troostiana,* genital anatomy showing secondary penial retractor muscle (spr); Fig. 37a, enlarged view of penis of the same specimen showing penial retractor muscle enveloping base of the vas deferens and section of the penis (the secondary penial retractor muscle is omitted for clarity); Fig. 37b, opened penis of the same specimen. Fig. 38, *Daedalochila fatigiata,* genital anatomy; Fig. 38a, penis of the same specimen opened to the vas deferens. Fig. 39, *D.*

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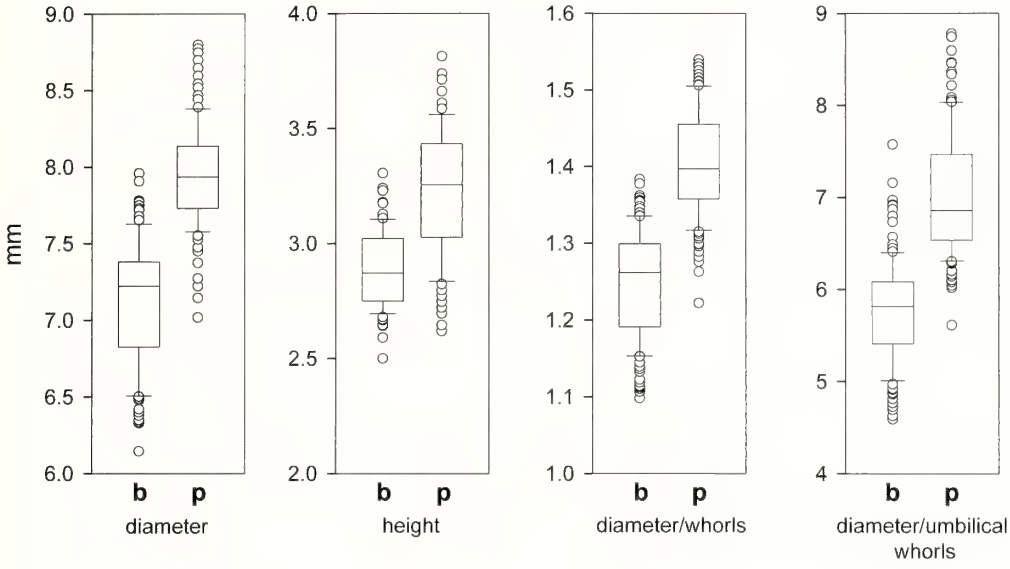

Figure 40. Box plots of variation of shell size and coiling in *Daedalochila bisontes* sp. nov. and *Daedalochila peregrina*. The boxes represent the 25th-75th percentiles of data and the bars the 10th-90th percentiles, data outside of these ranges are represented by circles, and the lines within the boxes illustrate the median. **b**, *D. bisontes*, **p**, *D. peregrina*. Student's t-test generates $p < 0.001$ for differences of the means of these measurements for the species pairs in all four graphs.

internal features, and the degree of development of the epiphallus, are given below and summarized in Table 2.

Daedalochila peregrina (Rehder, 1932)
(Figs. 26-28)

FMNH293230: Arkansas, Searcy County, Highway 65 opposite junction with Highway 74, approximately 6 miles NW of Marshall, B. Coles, 21 April 2002. Six specimens dissected, penis opened in 3 specimens.

The penis is approximately cylindrical, maximum width/length 0.15-0.19 (mean = 0.17, n = 3); the epiphallus short and ill defined (Fig. 26). The vas deferens is thickened proximally, maximally thickened at the junction with the prostate gland (approximately 2-3 times its minimum diameter). Internally, the penis bears two pilasters that run the entire length and which are developed into low (Fig. 27) or broad (Fig. 26a) fleshy ridges that are variously branched and anastomose. One pilaster passes into the vas deferens as a low ridge. No glandular or colored areas were visible at the magnification used (40X).

The penis differs from that of *Daedalochila bisontes* in being more uniformly cylindrical, with a short, ill-defined epiphallus and the pilasters being low, variously branched, and fused.

Daedalochila jacksoni (Bland, 1866)
(Figs. 29-30)

FMNH293236: Arkansas, Benton County, Hobbs Wildlife Management Area, G. Walsh, 26 January 2003. Three specimens dissected including opening of the penis.

The penis has a narrow base, widens at mid-length, and narrows before a distinct, swollen apex, maximum width/length 0.14-0.17 (mean = 0.15, n = 3) and does not possess an epiphallus (Figs. 29, 30). The vas deferens is thickened proximally, strongly (and maximally) thickened at the junction with the prostate gland (approximately 3-4 times the minimum diameter). Internally, the penis has two pilasters strongly developed at the penial apex into high, fleshy lamellae; apically, the pilasters appear to fuse with the walls of the vas deferens (Fig. 29a, 30a). No glandular or colored areas were visible at the magnification used (40X). The atrium was relatively longer than observed for *Daedalochila bisontes*, approximately 2/5 the length of the penis.

The penis differs from that of *Daedalochila bisontes* in

Figures 31-39. (continued)
fatigiata, distal genitalia of a second specimen; Fig. 39a, enlarged view of the penis of the same specimen showing penial retractor muscle enveloping the base of the vas deferens and section of penis (inset); Fig. 39b, penis of the same specimen showing strongly developed pilasters and apical complex (in a less disturbed state than in Fig. 38a); the features between the vas deferens and the apical penial complex of Fig. 39b are due to ripping of the tissues during dissection. Scale bars are all 1 mm and refer to the gross genitalia only. Abbreviations: ag, albumin gland; at, atrium; ep, epihallus; fo, free oviduct; fpsc, fertilization pouch-seminal receptacle complex; hd, hermaphrodite duct; p, penis; pep, puckered epiphallus at junction with penis; pr, penial retractor muscle; pt, prostate gland; s, spermatheca; spr, secondary penial retractor muscle; u, uterus; vd, vas deferens; vg, vagina.

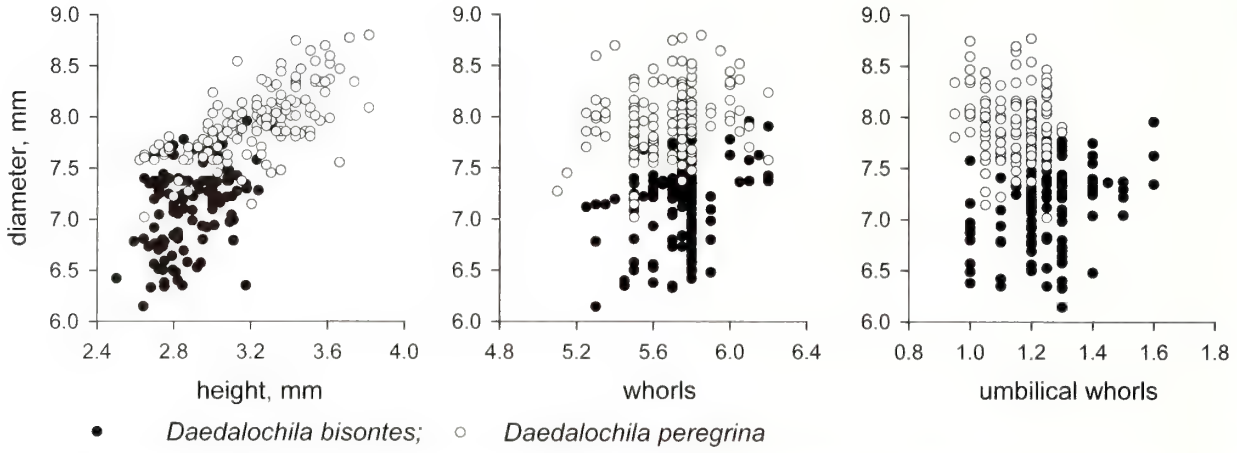

Figure 41. Discrimination of *Daedalochila bisontes* and *Daedalochila peregrina* on the basis of size and coiling. Shell diameter has been plotted against shell height, whorl number, and umbilical whorl number for each measured shell.

possessing a distinct, swollen apex corresponding to the strong development of the pilasters in the apex of the penis.

Daedalochila plicata (Say, 1821)
(Figs. 31-34)

FMNH293238: Alabama, Jackson County, County Road 33, approximately 5 miles E of Skyline, B. Coles, 18 April 1999. Three specimens dissected, including opening of the penis.

The penis is cylindrical, maximum width/length 0.11-0.12 (mean = 0.12, n =3), with a short epiphallus. The vas deferens is thickened proximally, reaching its maximum diameter (approximately 3 times its minimum diameter) at the junction with the prostate gland (Fig. 34). Internally, the penis bears two pilasters that are variously developed into low lamellae. Apically, the pilasters fuse with a pendant conical projection that accurately defines the beginning of the epiphallus (Figs. 31a-33). In detail, this consists of extension of the walls of the vas deferens into the penial lumen; the thickened walls being rolled inwards (Fig. 33) (note that in the less disturbed state, the in-rolled margins are in contact with each other). No glandular or colored areas were visible at the magnification used (40X). The atrium was relatively longer than in *Daedalochila bisontes* (approximately 1/2 the length of the penis) and the vagina was shorter.

Daedalochila plicata differs from all other species of the *Polygyra plicata* group by the presence of the apical pendant conical projection and the short but well-defined epiphallus.

Table 2. Summary of the morphology of the penis and vas deferens for the *Polygyra plicata* group[1] as observed by the authors.

	D. plicata	*D. bisontes*	*D. dorfeuilliana*	*D. peregrina*	*D. jacksoni*	*D. fatigiata*	*D. troostiana*
1. pendant conical projection in penis	present	absent	absent	absent	absent	complex	complex
2. penis width/length	~0.12	~0.12	~0.15	~0.17	~0.15	~0.34	~0.38
3. epiphallus	short, well-defined	variable	distinct, reflexed	short, ill-defined	absent	absent	absent
4. thickening of proximal vas deferens (vd)	~3X	~2-3X	~2-3X	~2-3X	~3-4X	~4X	~2X (thick throughout)
5. apex of penis	simple	variable	bent	simple	complex	simple, but enlarged	simple, but enlarged
6. pilasters	2	2	2	2	2	3	2
7. penial retractor muscle	apical	apical	apical	apical	apical	envelops distal vd	envelops distal vd

[1] All species of the group are here included in the genus *Daedalochila*.

Daedalochila dorfeuilliana (Lea, 1838)
(Figs. 35-36)

FMNH293240: Arkansas, Hempstead County, Lake Millwood at Saratoga Landing, B. Coles, 5 January 2003. Six specimens dissected, penis opened in 4 specimens. *D. dorfeuilliana sampsoni* (Wetherby, 1881). FMNH293241: Arkansas, Marion County, Buffalo Point, 3 miles E of Mull, B. Coles, 20 January 2003. One dissected including opening of the penis.

The penis is narrow basally, thickened in mid-region and contracted into a reflexed epiphallus, maximum width/length 0.09-0.19 (mean = 0.15, n =4). The vas deferens is thickened proximally, reaching its maximum diameter (approximately 2-3 times its minimum diameter) at the junction with the prostate gland. Internally, the penis bears two pilasters that are variably thickened into lamellae and lobes. These form low ridges basally and apically, and apically appear to extend into the epiphallus (Fig. 35a). One specimen had a short and stout epiphallus (Fig. 36). In this example, the pilasters were seen to be puckered apically and contiguous with the thickened and puckered tissues around the junction of the epiphallus and penial lumen. One fold of the puckered epiphallus was prominent, corresponding to the pilaster passing into the epiphallus.

The single specimen of the form *sampsoni* did not differ anatomically from the nominal form.

The general form of the penis of *Daedalochila dorfeuilliana* is similar to that of *Daedalochila bisontes*; however, the lobes of the pilasters are more strongly developed apically.

Daedalochila troostiana (Lea, 1839)
(Figs. 37, 37a, 37b)

FMNH293242: Tennessee, De Kalb County, Cove Hollow Road at N end of Center Hill Lake, B. Coles, 16 April 1999. One dissected including opening of the penis.

The penis is stout, narrower basally, thickened terminally, maximum width/length 0.38, without any trace of epiphallus. The penial retractor muscle is broad and envelops the penial apex and the penial end of the vas deferens (Fig. 37a). A very fine sheet-like secondary retractor muscle connects the terminal and basal penis (Fig. 37). The vas deferens is thickened throughout, becoming greatly thickened at the junction with the prostate gland (approximately twice its minimum diameter). Internally, the penis bears two pilasters that extend as low ridges into the vagina and atrium (Fig. 37b). Terminally, these fuse with a complex of folds and thickened tissue that is contiguous with the walls of the penis, and which appears to fuse with the thickened walls of the vas deferens (Fig. 37b). The atrium is relatively long, approximately half the length of the penis.

Daedalochila fatigiata (Say, 1829)
(Figs. 38-39)

FMNH293245: Tennessee, Perry County, Mousetail Landing State Park, Perryville, B. Coles, 24 March 2000. Four dissected, penis opened in two specimens. (The form is *Daedalochila fatigiata internuntia* [Pilsbry, 1940] on the basis of distinct rib striation below the periphery of the shell).

The penis is narrow basally, thickened terminally, maximum width/length 0.32-0.36 (mean = 0.34, n = 4), without any trace of epiphallus. The penial retractor muscle envelops the apex of the penis and the penial end of the vas deferens (Fig. 39a). The vas deferens widens proximally to reach its maximum diameter (approximately 4 times its original diameter) at the junction with the prostate gland. Internally the penis bears three pilasters that are narrow basally and become strong at mid-length where they are developed into lamellae. In the terminal penis, these fuse with a series of complex folds and thickened tissue that is contiguous with the penial walls, one part of which forms a prominent lobe; this complex appears to fuse with the thickened walls of the vas deferens (Figs. 38a, 39b). The atrium is relatively long, approximately half the length of the penis.

Daedalochila troostiana and *Daedalochila fatigiata* differ from other members of the *Polygyra plicata* group in the combination of a thicker penis that is markedly swollen at the apex, the complete lack of an epiphallus, the penial retractor muscle enveloping the vas deferens at its junction with the penis, and the complex series of folds and thickened tissue in the apical region of the penis.

DISCUSSION

The most recent studies of the Polygyridae are those of Emberton (1988, 1991, 1995), who, on the basis of detailed studies and summaries of previous work, established clades defined by shared characters regardless of their subsequent evolutionary modification. The resultant taxonomy differs in many respects from that of Pilsbry (1940) which, until that time, was the most authoritative study of the family. Pilsbry included all the depressed, umbilicate species of the Polygyridae in which the peristome is connected by a raised parietal callus or a V-shaped parietal lamella, into a single genus, *Polygyra* Say, 1818. The species having only a parietal lamella were placed in the subgenus *Polygyra sensu stricto* and the remaining species with parietal, basal, and palatal lamellae were placed in the subgenus *Daedalochila* Beck, 1837. Emberton (1995) treated *Polygyra*, *Daedalochila,* and Pilsbry's sections of *Daedalochila* (*Linisa* Pilsbry, 1930 and *Lobosculum* Pilsbry, 1930) at full generic status. However, Emberton's most substantive departure from Pilsbry's treatment was to include these four genera; *Praticolella* Martens, 1892; and *Giffordius* Pilsbry, 1930 in a tribe, the Polgyrini

sensu stricto, and to bring *Praticolella*, *Linisa* (in part), and *Lobosculum* (in part) together as a clade on the basis of the anatomy of the penis (a sacculate glandular diverticulum of the lower penis), despite their dissimilar shells. Pilsbry's (1940) concept of *Daedalochila* (with the exception of *Linisa* and *Lobosculum*, as mentioned above) was retained by Emberton in the form of a clade defined by "a vestigial epiphallus without a flagellum" and preceding characters that define the tribe (Emberton 1995: 77, characters 70-74, 86). The character states used by Emberton to define genera and subgenera within this clade and his assignment of species are summarized in Table 1. This arrangement is based on limited anatomical information. For *Millerelix*, anatomy has been illustrated only for *Millerelix* (*Millerelix*) *mooreana* (Pratt 1981a: 30, fig. 4), *Millerelix* (*Millerelix*) *dorfeuilliana* (Pratt 1981a: 44, fig. 8), and *Millerelix* (*Prattelix*) *plicata* (Emberton 1995: 80, fig. 4. (Pratt's figures 4 and 8 are also reproduced in Emberton 1995: 81, fig 5.) (Rehydrated material of *Millerelix* [*Millerelix*] *gracilis* [Hubricht, 1961] was also examined by Pratt [1981a].)

Pratt established *Millerelix* on the basis of the genital anatomy of *Millerelix mooreana* and *Millerelix dorfeuilliana* and, in fact, explicitly excluded *Polygyra plicata* from the genus (Pratt 1981a: 27). Emberton extended the concept to include all the members of the *Polygyra plicata* group because of the presence of "an apical, pendant, conical projection" in the penis of *Polygyra plicata*. Pratt did not indicate whether he observed this feature for *Polygyra plicata* and, if so, whether he regarded it as being homologous with that described in his diagnosis of *Millerelix* "the very short epiphallus enters the penis through a short tubular verge" (Pratt 1981a: 25). Emberton qualified his revised description of this diagnostic feature of *Millerelix* to say "and derivatives thereof" presumably to allow extension of Pratt's concept of *Millerelix* to include *Polygyra plicata*.

It is against this background that we compare our dissections of *Daedalochila plicata* and *Daedalochila dorfeuilliana* with published illustrations and discuss the newly presented anatomy of *Daedalochila bisontes, Daedalochila fatigiata, Daedalochila jacksoni, Daedalochila peregrina,* and *Daedalochila troostiana*.

Our dissections of *Daedalochila* (*Millerelix* [*Prattelix*]) *plicata* agree with that of Emberton (1995), both in form and dimensions, notably the relative width/length of the penis (0.12) and the thickening of the proximal vas deferens. The vas deferens at its junction with the prostate gland was approximately three times its minimum diameter in both Emberton's and our specimens; however, it appears more stout throughout in Emberton's illustration (Emberton 1995: 80, fig. 4). The apical, pendant, conical projection was a prominent feature of all specimens. This feature appears to be an extension of the vas deferens into the penial lumen and, although not tubular, otherwise appears to conform to Pratt's diagnosis of *Millerelix*.

In contrast, our dissections of *Daedalochila* (*Millerelix* [*Millerelix*]) *dorfeuilliana* bear little resemblance in detail to those of Pratt. Specimens that we dissected had shorter penises and the proximal vas deferens were thickened at their junctions with the prostate gland. The length of the penis in each of our specimens (approximately 2.5 mm) was almost half that of Pratt's specimen (4.0 mm), although the width (0.4 mm) was similar (Pratt, 1981a: 43). The internal anatomy of the penis agrees with Pratt's description in the presence of two longitudinal pilasters, but the diagnostic generic character of a *tubular verge* was not seen in any of our specimens. In fact, Pratt indicated that this is a variable feature in *Millerelix dorfeuilliana*, stating that "in a relaxed specimen . . . the verge is short, reduced to a p[a]pilla" (Pratt, 1981a: 43), even though for the same specimen it is illustrated as a prominent feature and drawn as if of greater size than in *Millerelix mooreana* (Pratt 1981a, figs. 4 and 8).

These data suggest that compared to Pratt's material, our material was subject to contraction during preservation (even though both sets of material were apparently preserved in a similar way); preservation method being known to result in changes in tissue dimensions (Emberton 1989). Nevertheless, because all our material was preserved in the same way, because museum material is usually preserved using the same method as that used by us, and because the anatomy of our specimens of *Daedalochila plicata* closely resemble that of Emberton (1995), we feel that comparisons within our material and with other published dissections are meaningful.

A comparison of the diagnostic generic and subgeneric characters within the *Daedalochila/Millerelix* clade as used by Emberton (1995) (Table 1) and as observed by us for the members of the *Polygyra plicata* group (Table 2) indicate the following:

1. *An apical pendant conical projection in the penis* was seen only in *Daedalochila plicata*, presents a different form in *Daedalochila troostiana* and *Daedalochila fatigiata*, was not confirmed for *Daedalochila dorfeuilliana*, and is absent in *Daedalochila bisontes, Daedalochila peregrina,* and *Daedalochila jacksoni*.
2. *Penis width/length.* For *Daedalochila plicata, Daedalochila bisontes, Daedalochila dorfeuilliana, Daedalochila peregrina,* and *Daedalochila jacksoni*, the width/length of the penis is within (or close to) the limit defining *Millerelix*. For *D. dorfeuilliana* (*Millerelix* [*Millerelix*]) the ratio is approximately twice than that given for *Millerelix* (*Millerelix*) and does not appear to be different from that of *D. plicata* (*Millerelix* [*Prattelix*]). For *Daedalochila troostiana* and

Daedalochila fatigiata, this ratio is considerably greater than that defined for *Millerelix*, greater than that for *D. plicata, D. bisontes, D. dorfeuilliana, D. peregrina,* and *D. jacksoni*, and similar to that for *Daedalochila (Upsilidon)*.

3. *Epiphallus*. An epiphallus is a well-defined feature of *Daedalochila plicata*, variable in development or ill-defined for *Daedalochila dorfeuilliana, Daedalochila bisontes* and *Daedalochila peregrina,* and absent in *Daedalochila jacksoni, Daedalochila troostiana,* and *Daedalochila fatigiata.*
4. *Thickness of proximal/distal vas deferens*. All species showed a thickened proximal vas deferens and this feature was not markedly different between *Daedalochila dorfeuilliana* (*Millerelix* [*Millerelix*]) and *Daedalochila plicata* (*Millerelix* [*Prattelix*]).
5. The *apex of the penis* is variable between species (and defined by the shape of the pilasters and other internal features).
6. The *penial pilasters* vary in number from two to three.
7. The *penial retractor muscle* envelops the distal vas deferens in *Daedalochila fatigiata* and *Daedalochila troostiana* (and a secondary retractor muscle is present in at least *D. troostiana*).

Thus, the characters used to define *Millerelix sensu* Emberton are not consistently present in the *Polygyra plicata* group and do not adequately address the variation between the species included by Emberton in *Millerelix*. Conclusions based on these new data and the published illustrations referred to above are as follows:

1. The presence of a pendant conical projection in the apical penis varies between the species, and/or appears to vary between individuals (*Daedalochila dorfeuilliana*), or is dependent on the methods of preservation (*D. dorfeuilliana*).
2. The width/length of the penis does not differentiate between *Millerelix* (*Millerelix*) (*Daedalochila dorfeuilliana*) and *Millerelix* (*Prattelix*) (*Daedalochila plicata*), and may be an unreliable character because of variation between individuals of the same species or contraction during preservation.
3. The thickening of the proximal epiphallus at its junction with the prostate gland does not differentiate between *Millerelix* (*Millerelix*) and *Millerelix* (*Prattelix*) and is a variable feature, possibly subject to differences between individuals of the same species and changes during preservation.
4. Several features of Pilsbry's *Polygyra plicata* group appear to be common to *Daedalochila sensu* Emberton, that is, the complete lack of an epiphallus in *Daedalochila jacksoni, Daedalochila troostiana,* and *Daedalochila fatigiata* and the stout penis of *D. troostiana* and *D. fatigiata.*

Because of this we feel that, when this novel anatomical information is included, the diagnostic characters of *Millerelix* and *Daedalochila* are shown to be unreliable for defining clades. Thus, the species included in *Millerelix sensu* Emberton (1995) should revert to the senior genus *Daedalochila* Beck, 1837. That is, the *Millerelix/Daedalochila* clade of Emberton (1995) is assigned the generic rank of *Daedalochila. Daedalochila* has been used in this sense in this study.

Despite this conclusion, it is not our intention to imply that *Daedalochila* should not be separated into multiple genera or subgenera. For example, *Daedalochila sensu stricto* and *Upsilidon* Pilsbry, 1940 were originally defined on the basis of shell characters, that is, the ear-shaped parietal lamella and raised parietal callus of *Daedalochila (Daedalochila)* (Pilsbry 1940: 592) and the U-shaped parietal lamella of *Daedalochila (Upsilidon)* (Pilsbry 1940: 637).

Several shell features that have potential as diagnostic subgeneric characters are also present in the *Polygyra plicata* group. For example, *Daedalochila bisontes, Daedalochila jacksoni,* and *Daedalochila peregrina* have in common a deeply immersed, long, and curved palatal lamella (Figs. 10-13); and *Daedalochila troostiana* and *Daedalochila fatigiata* are closely similar in apertural features (Figs. 15-16). However, the relationship between shell features and anatomical features within *Daedalochila* are poorly known and the species of the genus require further study before reliable subgeneric status can be assigned. Notably, the anatomical relationship of *D. troostiana* and *D. fatigiata* with *Upsilidon* should be examined, and the consistency of the internal features of the penis of *Daedalochila dorfeuilliana* and its relationship to *Daedalochila mooreana* need to be reexamined. Similarly, the newly observed anatomical features of Table 2 need to be included in any novel cladistic or systematic analyses of the genus.

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LITERATURE CITED

Coles, B. F. and G. E. Walsh, 1999. A revised list of Arkansas terrestrial mollusks with notes on the geographic distribution of species. *Journal of the Arkansas Academy of Sciences* **53**: 32-37.

Emberton, K. C. 1988. The genitalic, allozymic, and conchological evolution of the eastern North American Triodopsinae (Gastropoda: Pulmonata: Polygyridae). *Malacologia* **28**: 159-273.

Emberton, K. C. 1989. Retraction/extension and measurement errors in a land snail: Effects on systematic characters. *Malacologia* **31**: 157-173.

Emberton, K. C., 1991. The genitalic, allozymic and conchological evolution of the tribe Mesodontini (Pulmonata: Stylommatophora: Polygyridae). *Malacologia* **33**: 71-178.

Emberton, K. C. 1995. When shells do not tell; 145 million years of evolution in America's polygyrid land snails, with a revision and conservation priorities. *Malacologia* **37**: 69-110.

Hubricht, L. 1985. The distributions of the native land mollusks of the eastern United States. *Fieldiana*, Zoology (New Series)**24**: 1-191.

Pilsbry, H. A. 1939. *Land Mollusca of North America (North of Mexico)*, Vol. 1, Part 1. The Academy of Natural Sciences of Philadelphia, Philadelphia.

Pilsbry, H. A. 1940. *Land Mollusca of North America (North of Mexico)*, Vol. 1, Part 2. The Academy of Natural Sciences of Philadelphia, Philadelphia.

Pratt , W. L. 1981a. *A Revision of the Land Snail Genus* Polygyra *in Texas*. Ph.D. Dissertation, University of Arizona, Tucson.

Pratt, W. L. 1981b. A revision of the land snail genus *Polygyra* in Texas. *Dissertation Abstracts International* **42**: 1352-B.

Turgeon, D. D., J. F. Quinn Jr., A. E. Bogan, E. V. Coan, F. G. Hochberg, W. G. Lyons, P. M. Mikkelsen, R. J. Neves, C. F. E. Roper, G. Rosenberg, B. Roth, A. Scheltema, F. G. Thompson, M. Vecchione, and J. D. Williams. 1998. *Common and Scientific Names of Aquatic Invertebrates from the United States and Canada, Mollusks*, 2nd Ed. American Fisheries Society, Special Publication 26. American Fisheries Society, Bethesda, Maryland.

Walsh, G. E. and B. F. Coles, 2002. Distributions and geographical relationships of the polygyrid land snails (Mollusca, Gastropoda, Polygyridae) of Arkansas. *Journal of the Arkansas Academy of Sciences* **56**: 212-219.

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RESEARCH NOTE

Crepidula convexa Say, 1822 (Caenogastropoda: Calyptraeidae) in Washington State, U.S.A.

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Abstract: With the increasing attention to the expansion and impact of invasive species, it has become more important to document carefully new observations of introduced species. Here we document the occurrence of *Crepidula convexa*, a species from the north Atlantic, in Washington State, U.S.A. DNA sequence data suggest that the animals in Washington originated from the northern part of the species's native range.

Keywords: Gastropod taxonomy, cryptic species, introductions

Several species of calyptraeid gastropods (slipper limpets, cup-and-saucer limpets, and hat limpets) have successfully invaded new locales following human-mediated introductions. Two are now particularly widespread. *Crepidula fornicata* (Linnaeus, 1758), native to the northwest Atlantic, has invaded much of the northern coast of Europe since its introduction to England on oysters at the turn of the century (Blanchard 1997, Minchin *et al.* 1995). Its range now also extends into the Mediterranean (Zibrowius 1992). *Crepidula fornicata* was also introduced with shipments of the Atlantic oyster *Crassostrea virginica* (Gmelin, 1791) to Puget Sound and Willapa Bay in Washington State and Humboldt Bay, California, on the Pacific coast of North America (Carlton 1979, 1992, Wonham and Carlton 2005). *Crepidula onyx* Sowerby, 1824, a similar species native to southern California, has become widespread in Asia (Korea: Choe and Park 1992, Japan: Ekawa 1985, Hong Kong: Morton 1987) since it was first reported in Japan in 1968 (Woodruff *et al.* 1986).

Other introduced calyptraeids have more limited nonnative ranges. *Crepidula convexa* Say, 1822 is native to the Atlantic coast of North America from Nova Scotia to Georgia (Collin 2002). It was introduced to the Pacific coast in San Francisco Bay, California with shipments of Atlantic oysters (Carlton 1979, 1992, Wonham and Carlton 2005). The Atlantic species *Crepidula plana* Say, 1822 is similarly thought to have been introduced to Puget Sound, Willapa Bay, and San Francisco Bay (Carlton 1979, 1992, Wonham and Carlton 2005). *Bostrycapulus calyptraeformis* (Deshayes, 1830) has been introduced into Hawai'i, where it was reported as *Crepidula aculeata* (Gmelin, 1791) and an undetermined species of *Bostrycapulus* (also usually cited as *C. aculeata*) (Collin 2005) has recently become established in Alicante Harbor, Spain (Zibrowius 1992).

The apparent contained distributions of these species may reflect a truly limited establishment in the new region, or they may be artifacts of limited collection records or misidentifications as other species. Here we report an additional location in the North Pacific in which *Crepidula convexa* has become established. Given the complicated taxonomic history of calyptraeid gastropods and the large number of morphologically cryptic species, genetic analysis is particularly useful in confirming shell-based identifications and determining source populations for introduced species. We use morphological and genetic analysis to confirm the presence and source of *C. convexa* in Padilla Bay, northern Puget Sound, Washington, USA, and discuss the implications of these new records.

Animals of the genus *Crepidula* are found on the intertidal mudflats of Padilla Bay (48°28′N, 122°31′W; Fig. 1) almost exclusively as epibionts on the introduced Asian mudsnail *Batillaria cumingi* (Crosse, 1862) (previously referred to as *Batillaria attramentaria* [Sowerby, 1855] on the Pacific coast of North America) (O'Connor *et al.* 2002). Individuals were collected from *B. attramentaria* in March 2001 and preserved in 70% ethanol. Examination of their shell morphology and anatomy showed them to be morphologically indistinguishable from *Crepidula convexa*. The dark

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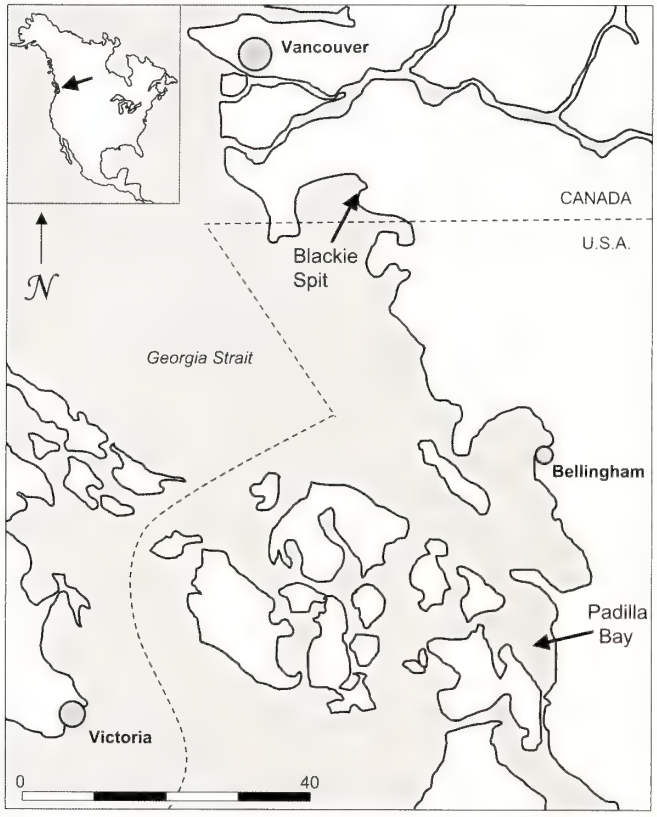

Figure 1. Collection sites for *Crepidula convexa*: Blackie Spit (49°04′N, 122°53′W), South Surrey, British Columbia, Canada, and Padilla Bay (48°28′N, 122°31′W), northern Puget Sound, Washington State, U.S.A. Scale in km.

Figure 2. Dorsal and ventral views of a shell of *Crepidula convexa* collected from the shell of an individual of *Batillaria cumingi* in Padilla Bay, March 2001. Shell length 12 mm.

grayish shells (Fig. 2) represent a morphology that is more common in populations of *C. convexa* from New England than in more southern populations of this species (Collin 2002). However, this morphology is also present in the southern populations of *C. convexa* and occurs in its sister species *C. ustulatulina* Collin, 2002. Therefore the samples from Washington could not be assigned to *C. convexa* with absolute certainty on the basis of their shells alone.

To confirm this identification genetically and to determine the source population, DNA was extracted from 2 specimens and 640 base pairs of cytochrome oxidase c subunit I (COI) were sequenced following the methods of Collin (2001). Neighbor-joining analysis of these new sequences (GenBank: AY615331, AY615332) in combination with sequences previously reported by Collin (2001) showed that the animals from Padilla Bay are indeed *Crepidula convexa* (Figure 3). The haplotypes from Padilla Bay are very similar to haplotypes of animals collected from Rhode Island and New Jersey. They clearly cluster with the samples from the northeastern United States to the exclusion of *C. convexa* from Georgia, as does a previously sequenced animal from Blackie Spit, British Colombia. Development was not observed.

It is unclear when *Crepidula convexa* first appeared in the Pacific Northwest. It may have been introduced with imports of Atlantic oysters, *Crassostrea virginica*, which were common between the 1870s and 1930s (Carlton 1992, Wonham and Carlton 2005). It was first observed in Washington State more than 40 years later in Padilla Bay in 1970 on shell surfaces of *Batillaria cumingi* (Penttila 1971 and D. Penttila personal communication) but was not reported in regional surveys of the same time period (Sylvester and Clogston 1958, Jeffrey 1976, Carlton 1979, 1992). Since 1999 it has been observed in Padilla Bay at densities of up to 30/m^2 (O'Connor *et al.* 2002). In British Columbia, *C. convexa* was first collected in 1988 at Blackie Spit in Boundary Bay (R. Forsyth *in* Carlton 1992, Collin 2001) (Figure 1). We are not aware of established populations elsewhere in the Pacific Northwest, including San Juan Island, Washington (M. Dethier, personal communication). It is not known whether the

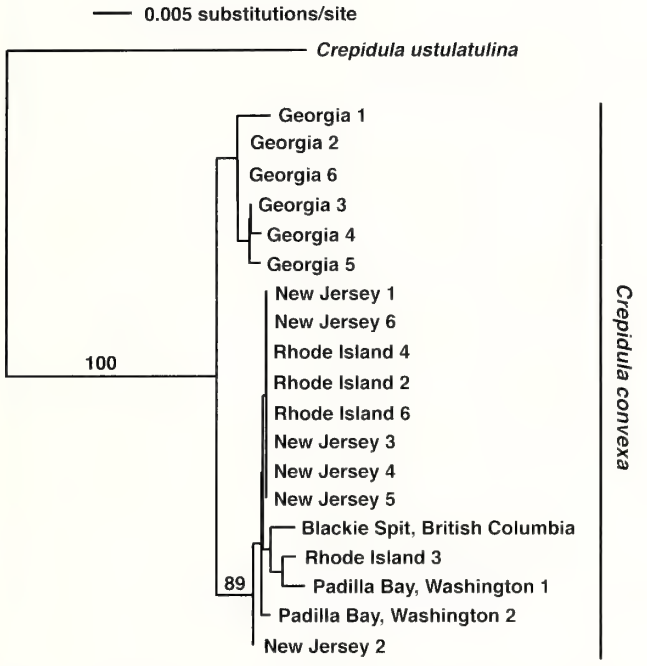

Figure 3. Neighbor-joining tree of 640 bp of COI sequence data from *Crepidula convexa*. Bootstrap percentages greater than 70% are indicated above the branches.

current populations of *C. convexa* in the Pacific Northwest represent the spread from a single introduction or sites of multiple introductions, since Atlantic oysters were planted in numerous locations throughout the region (Carlton 1979, Wonham and Carlton 2005).

Although identification of introduced calyptraeids has not always been clear-cut (e.g., the initial misidentification of *Crepidula onyx* as *Crepidula fornicata* in Asia), the situation on the west coast of North America is relatively straightforward. Members of the introduced species *C. fornicata* can be distinguished from members of the similar native species *C. onyx* on the basis of shell morphology and body pigmentation and is not easily confused with any other west coast species. Members of *Crepidula convexa* can also be distinguished from members of native Pacific species on the basis of morphology, but can be confused with both *Crepidula maculosa* (Conrad, 1846) and *Crepidula ustulatulina* from the southeast coast of North America. Neither of these species has been reported from the west coast of North America. The reports of introductions of *Crepidula plana* Say, 1822 are more difficult to verify. *Crepidula plana* is more or less morphologically indistinguishable from its close relative from the Atlantic *Crepidula depressa* Say, 1822 (Collin 2000), although this species generally occurs in warmer water. Additionally, *C. plana* cannot be distinguished from the Pacific species *Crepidula perforans* Valenciennes, 1846, *Crepidula williamsi* Coe, 1947, *Crepidula fimbriata* Reeve, 1859, and *Crepidula explanata* Gould, 1853 or from any of the flat white species of *Crepidula* from other parts of the world with any certainty without the aid of developmental and DNA sequence data. There are no reported introductions of calyptraeids on the Atlantic coast of North America.

ACKNOWLEDGMENTS

We are grateful to M. O'Connor, S. Riggs, and C. Harley for collection assistance and to J. T. Carlton for initial morphological identification of *Crepidula convexa* from Padilla Bay and J. T. Carlton and D. Franz for helpful comments.

REFERENCES

Blanchard, M. 1997. Spread of the slipper limpet *Crepidula fornicata* (L. 1758) in Europe: Current state and consequences. *Scientia Marina* **61** (Supplement 9): 109-118.

Carlton, J. T. 1979. History, Biogeography, and Ecology of the Introduced Marine and Estuarine Invertebrates of the Pacific Coast of North America. Ph. D. thesis. University of California, Davis.

Carlton, J. T. 1992. Introduced marine and estuarine mollusks of North America: An end-of-the-20th-century perspective. *Journal of Shellfish Research* **11**: 489-505.

Choe, B. L. and J. K. Park. 1992. Nine unrecorded mesogastropodous species (Gastropoda: Mollusca) from Korean waters—superfamilies Turritellacea, Calyptraeacea, Cypraeacea, and Tonnacea. *Korean Journal of Malacology* **8**: 29-40.

Collin, R. 2000. Phylogeny of the *Crepidula plana* (Gastropoda: Calyptraeidea) cryptic species complex in North America. Canadian Journal of Zoology **78**: 1500-1514.

Collin, R. 2001. The effects of mode of development on phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae). *Molecular Ecology* **10**: 2249-2262.

Collin, R. 2002. Another last word on *Crepidula convexa* and a description of *C. ustulatulina* sp. nov. (Gastropoda: Calyptraeidae) from the Gulf of Mexico. *Bulletin of Marine Science* **70**: 177-184.

Collin, R. 2005. Development, phylogeny, and taxonomy of *Bostrycapulus* (Caenogastropoda: Calyptraeidae), an ancient cryptic radiation. *Zoological Journal of the Linnean Society* **144**: 75-101.

Ekawa, K. 1985. Distribution and dispersion of *Crepidula onyx* in Japan. *Chiribotan* **16**: 37-44.

Jeffrey, R. 1976. A preliminary inventory of the biota of Padilla Bay. Washington State Department of Game. Reprinted October 1990 as *Padilla Bay National Estuarine Research Reserve Reprint Series* No. 1, Mount Vernon, Washington. 38 pp.

Minchin, D., D. McGrath, and C. B. Duggan. 1995. The slipper limpet, *Crepidula fornicata* (L.), in Irish waters, with a review of its occurrence in the north-eastern Atlantic. *Journal of Conchology* **35**: 247-254.

Morton, B. 1987. Recent marine introductions into Hong Kong. *Bulletin of Marine Science* **41**: 503-513.

O'Connor, M., M. Wonham, and C. Harley. 2002. Quantifying the impacts of an invader: The Asian mud snail *Batillaria attramentaria* on the mud flats of Padilla Bay, Washington. Washington State Department of Ecology (Publication No. 02-06-016). Available as *Padilla Bay National Estuarine Research Reserve Technical Report* No. 25, Mount Vernon, Washington.

Penttila, D. 1971. Introduced marine mollusks of Washington and Oregon: A critical essay in partial fulfillment of the requirements for a Master of Science Degree in Biology. Unpublished document available at Oregon Institute of Marine Biology Library.

Sylvester, R. O. and F. L. Clogston. 1958. A study of the preoperational marine environment in the vicinity of the Texas Company Refinery Puget Sound Works, Anacortes, Washington, for the Texas Company. Seattle. Available from the Library of the University of Washington, Seattle, Washington, and summarized in Jeffrey (1976).

Woodruff, D. S., L. L. McMeekin, M. Mulvey, and M. P. Carpenter. 1986. Population genetics of *Crepidula onyx*: Variation in a Californian slipper snail recently established in China. *The Veliger* **29**: 53-63.

Wonham, M. J. and J. T. Carlton. 2005. Trends in marine biological invasions at local and regional scales: The Northeast Pacific Ocean as a model system. *Biological Invasions* **7**: 369-392.

Zibrowius, H. 1992. Ongoing modifications of the Mediterranean marine fauna and flora by the establishment of exotic species. *Mésogée* **51**: 83-107.

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BOOK REVIEW

Field Guide to the Land Snails and Slugs of Eastern South Africa by Dai Herbert and Dick Kilburn (2004). Natal Museum, Pietermaritzburg. 336 pp. ISBN: 0-620-32415-5.

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There are many approaches to producing field guides. For birds, the Peterson format has proven highly successful; for plants, approaches based on taxonomy, or flower color, or form have been successful. For terrestrial molluscs, less popular than these more "charismatic" groups, no set format has become the standard; in fact only a few field guides are available. The two extremes of approaches may range from the highly detailed and rigorously taxonomically-oriented to the picture book. The field guide to the land snails of Britain and north-west Europe by Kerney and Cameron (1979) perhaps leans towards the former, with its inclusion of synonymies, dependence on dissection of the reproductive tract to distinguish species within some groups, and standardized drawings to illustrate species; it is a landmark work among molluscan field guides. However, field guides are not revisionary monographs, yet when they list synonymies or even introduce synonymies or new genus-species combinations or provide detailed descriptions of internal anatomy, they are coming close to the realm of revisionary works. The other extreme has probably not yet been exemplified among molluscan field guides, but the guide to the snails of Britain and Europe by Pfleger and Chatfield (1983) provides an illuminating comparison to Kerney and Cameron (1979), as it covers essentially the same fauna, but feels much more like a picture book, with its beautiful photographs rather than drawings, and is not as comprehensive. Photographs can often be more useful than drawings to the uninitiated, although necessarily cannot be generalized to avoid reflection of individual quirks of variation.

There are few field guides of non-marine molluscs other than these exemplary contributions from Europe; those for the land snails of Bali (Vermeulen and Whitten 1998), for British Columbia (Forsyth 2004) and for the introduced species in New Zealand (Barker 1999) spring to mind, although the last is more of a monograph than a field guide. This new field guide by Herbert and Kilburn is a major contribution to the genre that spans the range from the dry taxonomic to the picture book. The illustrations, mostly photographs, many of the living animals, and including SEMs of smaller species, but also drawings, are spectacular; you should get this book just to skim the pages and wonder at the diversity of terrestrial molluscan form and beauty. Yet as well as being a marvelous picture book, it is for the most part taxonomically rigorous and honest, to the extent that on the one hand detailed nomenclatural issues that only a trained taxonomist would be likely to understand are discussed (e.g., *Trachycystis watsoni*), but on the other when the taxonomy of the species has not been worked out (e.g., some of the species of *Gulella*) or species have not been identified (e.g., the introduced species of *Deroceras*) this uncertainty is explicitly stated.

The book is more than just a field guide. There are nine chapters preceding the actual family-by-family field guide. These deal with: the basic biology of snails and slugs (unfortunately but perhaps necessarily depending too much on the introduced *Helix aspersa*); the geology, climate, and vegetation of eastern South Africa (rather too much dry detail for the book's purpose); biogeography (essentially descriptive rather than synthetic); conservation (a good brief statement); the history of snail collecting and malacology in eastern South Africa (interesting, especially given that many of the known localities are attributable only to historical collections and because many species carry the names of the various collectors); snail collecting and preservation; how to use the field guide (one of the few places I have come across definitions, or at least illustrations of what it means when a shell is described as "ovate," "biconical," "subglobose," etc.); and classification (which frustratingly insists on using the paraphyletic "Prosobranchia"). The book ends with chapters on snail farming, snails and slugs as pests, a glossary, and an up to date, though short, list of further reading.

But the meat of the book is the over 200 pages treating the almost 300 species known from the relatively small region of eastern South Africa, essentially Kwazulu-Natal from

the Mozambique and Swaziland borders south to the former Transkei (about 800 km), and inland 100-200 km, for an area of about 120,000 km^2. Obviously, this limited geographic scope means that the book is of somewhat local interest as an actual field guide. However, many of the species ranges extend further into South Africa and the surrounding (or surrounded) south-east African countries of Lesotho, Mozambique, Swaziland, and Zimbabwe, if not further, and the book will be important to those studying the snails and slugs of the broader region. But to any land snail enthusiast (amateur or professional alike), regardless of their specific interest, the book will be a delight. Each species is described briefly—essentially a diagnosis—and illustrated with photographs of shells and often of the live animals—often more than one per species; these illustrations, which cover the pages, are what really makes this book so enjoyable. Distribution details are given, with maps of known localities. These maps are small and give point localities not ranges; given the small region covered by the field guide and the limited previous collecting, the maps may not be of much help to the field collector, who might legitimately expect to find any species just about anywhere within the region. Some information is also given about habitat and habits.

If I had to quibble, I would say that I sometimes found the writing a little colloquial, but then the book is supposed to be directed at the interested non-scientist (although professional scientists will also delight in it), so I suppose that's all right. Use of common names for families (alone rather than in conjunction with the scientific names in the general sections) might sometimes be frustrating for the professional malacologist who might know the scientific name but not the common name—I had no clue what an "awl snail" was, but I'm surrounded by subulinids! And finally, although the book is nice to look at and very readable, the cover of my copy did not seem particularly well attached to the pages in between.

In summary, this book sets a standard of accessibility and aesthetics for land snail field guides. I encourage everyone with an interest in land snails to get it. And if you are not particularly interested in land snails, you should also get it because it will show you what you are missing.

LITERATURE CITED

Barker, G. M. 1999. *Fauna of New Zealand. Number 38. Naturalised Terrestrial Stylommatophora (Mollusca: Gastropoda).* Manaaki Whenua Press, Lincoln, Canterbury, New Zealand.

Forsyth, R. G. 2004. *Land Snails of British Columbia.* Royal British Columbia Museum, Victoria.

Kerney, M. P. and R. A. D. Cameron. 1979. *A Field Guide to the Land Snails of Britain and North-West Europe.* Collins, London.

Pfleger, V. and J. Chatfield. 1983. A *Guide to the Snails of Britain and Europe.* Hamlyn, London.

Vermeulen, J. J. and A. J. Whitten. 1998. *Fauna Malesiana Guide to the Land Snails of Bali.* Backhuys Publishers, Leiden.

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The 72nd annual meeting of the American Malacological Society and the 39th annual meeting of the Western Society of Malacologists will be held jointly in Seattle from 29 July-3 August 2006 under the coordination of co-president Dr. Roland C. Anderson. The main venue for the meeting will be the University of Washington with reasonably-priced housing available at the University dormitories and the University Inn Motel. The opening night reception will be in the Burke Museum, located on campus, and the ending banquet will be at the university's University Club, also on campus. Thursday 3 August will be devoted to field trips.

There will be three symposia: one on cephalopod behavior organized by Jennifer Mather of the University of Lethbridge, one on chitons organized by Douglas Eernisse of the California State University at Fullerton, and one on opisthobranchs organized by Sandra Millen of the University of Bristish Columbia.

There will be a sale of molluscan reprints to benefit the student fund of the WSM and the traditional spirited auction of books and molluscan memorabilia (no shells) that will benefit the student funds of both organizations. Several notable items of cephalopod art have already been donated and a copy of Abbott's 2nd edition of American Seashells. Let's bring some of your unused reprints, books and molluscan art to benefit this very worthy cause! Reprints and auction items can be sent to Roland Anderson at the address below.

For further information please contact AMS and WSM president:

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Seattle, WA 98101 USA

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Roland.anderson@seattle.gov

AMERICAN MALACOLOGICAL SOCIETY, INC
FINANCIAL REPORT
General Accounts
2002 Income and Expenses

TOTAL ASSETS (January 1, 2002)			**$149,214.42**
INCOME			**$17,674.32**
Membership Dues		**8,680.00**	
Membership Dues (2000)	**130.00**		
Membership Dues (2001)	**1,047.00**		
Membership Dues (2002)	**7,433.00**		
Membership Dues (2003)	**70.00**		
Interest and Dividends from Endowment		**3,865.32**	
Life Membership Fund	**163.18**		
Symposium & Student Fund	**3,580.58**		
Money Market Interest	**73.20**		
Endowment Capital Gains	**48.36**		
Publications Income		**2,353.00**	
AMB Subscriptions	**1,085.00**		
AMB Page Charges	**996.00**		
AMB Back Issues	**79.00**		
AMB Reprint Charges	**108.00**		
AMB Postage & Misc. Income	**85.00**		
Donations		**2,776.00**	
Symposium Endowment Fund	**20.00**		
Student Endowment Fund	**2,756.00**		
Income from Annual Meeting		**0.00**	
EXPENSES			**$21,577.29**
Treasurer and Secretary Office Expenses		**610.07**	
Affiliate Memberships		**270.00**	
Banking & Credit Card Fees		**528.74**	
Incorporation & Registration Fees		**55.00**	
Insurance/Bond Fees		**505.00**	
Annual Meeting & Symposium Expenses		**10,009.00**	
Publication Expenses		**608.55**	
AMB (no volume published in 2002)	**500.16**		
Brochure printing	**108.39**		
Student Research Grants		**2,385.00**	
Travel Expenses for Officers		**5,605.93**	
Student Paper Awards		**1,000.00**	
NET LOSS IN 2002			**$3,902.97**
TOTAL ASSETS (December 31, 2003)			**$134,352.60**

****Includes capital gains and losses in endowment portfolios which fluctuate with the market.**

AMERICAN MALACOLOGICAL SOCIETY, INC
FINANCIAL REPORT
General Accounts
2003 Income and Expenses

TOTAL ASSETS (January 1, 2003)			**$134,352.60**
INCOME			**$45,433.15**
Membership Dues		**23,579.00**	
Membership Dues (2000)	**40.00**		
Membership Dues (2001)	**175.00**		
Membership Dues (2002)	**2,267.00**		
Membership Dues (2003)	**13,545.66**		
Membership Dues (2004)	**4,464.64**		
Membership Dues (2005)	**3,086.70**		
Interest and Dividends from Endowment		**3,374.43**	
Life Membership Fund	**184.80**		
Symposium & Student Fund	**3,189.63**		
Publications Income		**4,221.16**	
AMB Subscriptions	**1,470.00**		
AMB Page Charges	**367.00**		
AMB Back Issues	**840.00**		
AMB Reprint Charges	**650.00**		
AMB Postage & Misc. Income	**894.16**		
Donations		**3,897.00**	
Symposium Endowment Fund	**55.00**		
Student Endowment Fund	**2,644.75**		
Income from Annual Meeting		**11,558.81**	
EXPENSES			**$26,024.67**
Treasurer and Secretary Office Expenses		**882.30**	
Affiliate Memberships		**100.00**	
Banking & Credit Card Fees		**516.03**	
Incorporation & Registration Fees		**24.97**	
Insurance/Bond Fees		**500.00**	
Annual Meeting Deposit & Symposium Expenses		**5,691.00**	
Publication Expenses		**15,055.32**	
AMB (17-1/2)	**12,998.90**		
Reprints	**1,055.44**		
Managing Editor Travel	**1,000.98**		
Student Research Grants		**1,700.00**	
Travel Expenses for Officers		**1,055.05**	
Student Paper Awards		**500.00**	
NET GAIN in 2003			**$19,408.48**
TOTAL ASSETS (December 31, 2003)			**$165,457.02**

****Includes capital gains and losses in endowment portfolios which fluctuate with the market.**

AMERICAN MALACOLOGICAL SOCIETY, INC
FINANCIAL REPORT
General Accounts
2004 Income and Expenses

TOTAL ASSETS (January 1, 2004)			**$165,457.02**
INCOME			**$47,222.00**
Membership Dues		**11,936.00**	
Membership Dues (2001)	**40.00**		
Membership Dues (2002)	**435.00**		
Membership Dues (2003)	**1,293.00**		
Membership Dues (2004)	**8,288.00**		
Membership Dues (2005)	**1,350.00**		
Membership Dues (2006)	**530.00**		
Interest and Dividends from Endowment		**3,409.80**	
Life Membership Fund	**255.18**		
Symposium & Student Fund	**3,154.62**		
Publications Income		**11,202.84**	
AMB Subscriptions	**6,701.00**		
AMB Page Charges	**3,153.50**		
AMB Back Issues	**58.00**		
AMB Reprint Charges	**990.40**		
AMB Postage & Misc. Income	**299.94**		
Donations		**3,897.00**	
Symposium Endowment Fund	**57.50**		
Student Endowment Fund	**3,839.50**		
Income from Annual Meeting		**16,767.36**	
Other Misc. Income		**9.00**	
EXPENSES			**$48,322.50**
Treasurer and Secretary Office Expenses		**654.39**	
Recognition Plaques		**64.69**	
Affiliate Memberships		**200.00**	
Banking & Credit Card Fees		**405.14**	
Incorporation & Registration Fees		**25.00**	
Insurance/Bond Fees		**509.00**	
Annual Meeting Deposit & Symposium Expenses		**10,421.00**	
Publication Expenses		**30,500.66**	
AMB (18-1/2; 19-1/2)	**26,392.53**		
Reprints	**2,851.89**		
Managing Editor Travel	**1,256.24**		
Student Research Grants		**2,500.00**	
Travel Expenses for Officers		**2,542.62**	
Student Paper Awards		**500.00**	
NET LOSS in 2004			**$1,100.50**
TOTAL ASSETS (December 31, 2004)			**$170,765.82**

****Includes capital gains and losses in endowment portfolios which fluctuate with the market.**

INDEX TO VOLUME 21

AUTHOR INDEX

Aldana-Aranda, D. 21: 93
Avila-Poveda, O. H. 21: 93
Baqueiro-Cárdenas, E. R. 21: 93
Barr, K. R. 21: 113
Bennett, W. A. 21: 11
Bryce, T. D. 21: 31
Chávez, E. A. 21: 51
Coles, B. F. 21: 99
Collin, R. 21: 113
Cowie, R. H. 21: 117
Dimock, R. V. Jr. 21: 23
Fangue, N. A. 21: 11
Fisher, G. R. 21: 23
Gilbertson, L. H. 21: 17
Hanlon, S. D. 21: 45
Keferl, E. P. 21: 31
Koetsier, P. 21: 77
Lysne, S. 21: 77
Michel-Morfín, J. E. 21: 51
Mummert, A. 21: 1
Neves, R. J. 21: 1, 45
Newcomb, T. J. 21: 1
Parker, B. 21:1
Power, A. J. 21: 39
Radke, W. R. 21: 17
Singh, A. 21: 87
Singh, D. 21: 87
Singh, S. K. 21: 87
Stewart, T. W. 21: 59
Sukkestad, K. E. 21: 31
Tiffany, B. N. 21: 11
Walker, R. L. 21: 39
Walsh, G. E. 21: 99
Wonham, M. J. 21: 113
Yadav, R. P. 21: 87

PRIMARY MOLLUSCAN TAXA INDEX

[first occurrence in each paper recorded, new taxa in bold]

Acella 21: 65
aculeata, Crepidula 21: 113
acuminata, Lymnaea 21: 87
acuta, Physa 21: 36
acuta, Physella 21: 68
acuta, Pleurocera 21: 65
Agropecten 21: 42
Alasmidonta 21: 33
americanus, Modiolus 21: 41
Anadara 21: 39
anatine, Physella 21: 68
anceps, Helisoma 21: 69
ancillaria, Physella 21: 67
Ancylidae 21: 71
Ancylus 21: 71
angustata, Elliptio 21: 33
Anodonta 21: 33
antipodarum, Potamopyrgus 21: 78
Antodontinae 21: 33
antrosa, Helisoma 21: 69
antrosum, Helisoma 21: 69
antrosus, Planorbis 21: 69
Aplexa 21: 69
Arcidae 21: 42
arenaria, Mya 21: 23
armigera, Planorbella 21: 70
armigera, Planorbis 21: 70
armigera, Planorbula 21: 70
armigera, Segmentina 21: 70
armigerus, Planorbis 21: 70
aspersa, Helix 21: 117
Astyris 21: 41
Atrina 21: 12
attramentaria, Batillaria 21: 113
baboquivariensis, Sonorella 21: 22
barbadensis, Fissurella 21: 95
Batillaria 21: 96, 113
Bellamya 21: 62
bicarinata, Valvata 21: 36, 61
bicarinatus, Helisoma 21: 69
bicarinatus, Planorbis 21: 69
binneyi, Planorbis 21: 69
***binneyi**, Sonorella* 21: 17
bisontes, Daedalochila 21: 100
Bostrycapulus 21: 113
bowiensis, Sonorella 21: 21
bruneauensis, Pyrgulopsis 21: 82
Bulimnaea 21: 66
bulimoides, Fossaria 21: 66
bulimoides, Galba 21: 66
Busycon 21: 39, 96
Busycotypus 21: 39
Caenogastropoda 21: 93
californica, Cerithidea 21: 96
Calliostoma 21: 96
calyptraeformis, Bostrycapulus 21: 113
campanulata, Helisoma 21: 69
campanulata, Planorbella 21: 69
campanulatum, Helisoma 21: 69
campanulatum, Planorbella 21: 69
campanulatus, Planorbella 21: 69
campanulatus, Planorbis 21: 69
Campeloma 21: 36, 62
canaliculatus, Busycotypus 21: 39
caperata, Galba 21: 67
caperata, Limnaea 21: 67
caperata, Limnophysa 21: 67
caperata, Lymnaea 21: 67
caperata, Stagnicola 21: 67
carica, Busycon 21: 39
cariosa, Lampsilis 21: 33
carolinianus, Uniomerus 21: 33
cataracta, Pyganodon 21: 33
catascopium, Galba 21: 67
catascopium, Lymnaea 21: 67
catascopium, Stagnicola 21: 67
catus, Conus 21: 96
Cerithidea 21: 96
chinensis, Bellamya 21: 62
chinensis, Cipangopaludina 21: 62
chinensis, Viviparius 21: 62
cincinnatiensis, Pomatiopsis 21: 64
Cipangopaludina 21: 62
circumstriatus, Gyraulus 21: 69

clenchi, *Patera* 21: 105
coarctatum, *Campeloma* 21: 62
complanata, *Elliptio* 21: 6, 33
concholepas, *Concholepas* 21: 56
congaraea, *Elliptio* 21: 33
contectoides, *Viviparia* 21: 62
contectoides, *Viviparius* 21: 62
contrarium, *Busycon* 21: 96
Conus 21: 96
convexa, *Crepidula* 21: 113
Corbicula 21: 1, 35
corona, *Melongena* 21: 14
coronata, *Runcina* 21: 96
couperiana, *Anodonta* 21: 33
Crassostrea 21: 113
crassulum, *Campeloma* 21: 62
Crepidula 21: 113
crystallensis, *Stagnicola* 21: 67
cubicoides, *Goniobasis* 21: 65
cumingi, *Batillaria* 21: 113
Daedalochila 21: 99
dalli, *Galba* 21: 66
dalli, *Lymnaea* 21: 66
decidiosa, *Limnaea* 21: 66
decisa, *Campeloma* 21: 62
decisa, *Melantho* 21: 63
decisa, *Paludina* 21: 63
decisa, *Vivipara* 21: 63
decisum, *Campeloma* 21: 62
deflectus, *Gyraulus* 21: 69
deflectus, *Planorbis* 21: 69
delumbis, *Villosa* 21: 33
depressa, *Amnicola* 21: 64
depressa, *Crepidula* 21: 115
depressa, *Somatogyra* 21: 64
depressus, *Somatogyrus* 21: 64
Deroceras 21: 117
desidiosa, *Limnaea* 21: 66
desidiosa, *Limnophysa* 21: 66
diaphanous, *Ancylus* 21: 71
diaphanus, *Laevapex* 21: 71
dilatatus, *Menetus* 21: 69
dilatatus, *Micromenetus* 21: 69
dilatatus, *Planorbis* 21: 69
dorfeuilliana, *Daedalochila* 21: 100
dorfeuilliana, *Polygyra* 21: 99
Dreissena 21: 1
dubium, *Pisidium* 21: 35
Elimia 21: 65
elliptica, *Physella* 21: 67
Elliptio 21: 6, 33
elodes, *Galba* 21: 67
elodes, *Limnaeus* 21: 67
elodes, *Lymnaea* 21: 67
elodes, *Stagnicola* 21: 67
elongata, *Aplexa* 21: 69
elongates, *Limneus* 21: 67
elongates, *Lymneus* 21: 67
emarginata, *Cincinnatia* 21: 64
emarginata, *Galba* 21: 67
emarginata, *Lymnaea* 21: 67
emarginata, *Probythinella* 21: 64
emarginata, *Stagnicola* 21: 67
euglyptum, *Calliostoma* 21: 96
exacuous, *Menetus* 21: 70
exacuous, *Promenetus* 21: 70
exacutus, *Menetus* 21: 70
exacutus, *Planorbis* 21: 70
exigua, *Fossaria* 21: 66
exilis, *Campeloma* 21: 62
exilis, *Galba* 21: 67
exilis, *Lymnaea* 21: 67
exilis, *Stagnicola* 21: 67
explanata, *Crepidula* 21: 115
exustus, *Indoplanorbis* 21: 87
fasciola, *Lampsilis* 21: 3, 45
Fasciolaria 21: 14
fatigata, *Polygyra* 21: 99
Ferrissia 21: 36, 71
ferruginea, *Runcina* 21: 96
fimbriata, *Crepidula* 21: 115
Fissurella 21: 95
floridiana, *Anadara* 21: 39
fluminea, *Corbicula* 21: 1, 35
folliculata, *Elliptio* 21: 33
fornicata, *Crepidula* 21: 113
Fossaria 21: 66
fragilis, *Ferrissia* 21: 71
fusca, *Ferrissia* 21: 71
Fusconaia 21: 32
fuscus, *Ancylus* 21: 71
fuscus, *Laevapex* 21: 71
Galba 21: 66
galbana, *Galba* 21: 66
Gastropoda 21: 102
georgianus, *Viviparius* 21: 62
gibbus, *Agropecten* 21: 42
Giffordius 21: 109
gigas, *Strombus* 21: 93
Goniobasis 21: 36, 65
granosa, *Anadara* 21: 43
granulatisima, *Sonorella* 21: 21
Gulella 21: 117
Gundlachia 21: 71
Gyraulus 21: 69
gyrina, *Physella* 21: 67
halcyon, *Purgulopsis* 21: 36
haldemani, *Acella* 21: 65
halei, *Physella* 21: 68
Haliotis 21: 93
Helicoidea 21: 18
Helisoma 21: 69
Helix 21: 117
Helminthoglyptidae 21: 17
heterostropha, *Physella* 21: 68
hildrethiana, *Physella* 21: 67
hirsutus, *Gyraulus* 21: 69
humilis, *Fossaria* 21: 66
humilis, *Galba* 21: 66
humilis, *Limnaea* 21: 66
humilis, *Limnophysa* 21: 66
humilis, *Lymnaea* 21: 66
hunteria, *Fasciolaria lilium* 21: 14
Hydrobiidae 21: 64
hypnorum, *Aplexa* 21: 69
icterina, *Elliptio* 21: 33
idahoensis, *Pyrgulopsis* 21: 77
Ilyanassa 21: 96
imbecillis, *Utterbackia* 21: 23, 33
imperialis, *Sonorella* 21: 21
Indoplanorbis 21: 87
integer, *Somatogyrus* 21: 64
integra, *Campeloma* 21: 62
integra, *Cincinnatia* 21: 64
integra, *Melantho* 21: 63
integra, *Paludina* 21: 63
integra, *Physella* 21: 68
integrum, *Campeloma* 21: 62
internuntia, *Daedalochila fatigata* 21: 100
intertexta, *Viviparia* 21: 62
intertextus, *Viviparius* 21: 62
iowaensis, *Galba* 21: 67
iris, *Vilosa* 21: 3, 48
isogona, *Somatogyra* 21: 64
isogonus, *Somatogyrus* 21: 64
jacksoni, *Daedalochila* 21: 100
jacksoni, *Polygyra* 21: 99
jenksii, *Planorbula* 21: 70
joubini, *Octopus* 21: 11

judayi, *Cincinnatia* 21: 64
kamtschatkana, *Haliotis* 21: 96
kirtlandi, *Ferrissia* 21: 71
kirtlandiana, *Galba* 21: 67
lacustris, *Probythinella* 21: 64
Laevapex 21: 71
laevigata, *Haliotis* 21: 93
Lampsilinae 21: 33
Lampsilis 21: 3, 33, 45
lapidaria, *Pomatiopsis* 21: 64
lateralis, *Musculus* 21: 41
lentus, *Planorbis* 21: 69
Levapex 21: 36
lewisi, *Valvata* 21: 61
Limnaea 21: 66
Limnaeus 21: 67
Limnea 21: 67
Limneus 21: 67
Limnophysa 21: 66
limosa, *Amnicola* 21: 64
limosus, *Amnicola* 21: 64
limum, *Campeloma* 21: 36
Linisa 21: 105
Lioplax 21: 63
lithica, *Daedalochila* 21: 105
littorea, *Littorina* 21: 96
Littorina 21: 96
livescens, *Elimia* 21: 65
livescens, *Goniobasis* 21: 65
Lobosculum 21: 109
lordi, *Physella* 21: 67
lugubris, *Elliptio* 21: 33
lunata, *Astyris* 21: 41
lustrica, *Amnicola* 21: 64
lustrica, *Marstonia* 21: 64
lustrica, *Pyrgulopsis* 21: 64
Lymnaea 21: 66, 87
Lymnaeidae 21: 65
Lymneus 21: 67
maculosa, *Crepidula* 21: 115
magnifica, *Tulotoma* 21: 83
maleatus, *Viviparius* 21: 62
Margaritifera 21: 48
margaritifera, *Margaritifera* 21: 48
Marstonia 21: 64
masoni, *Fusconaia* 21: 32
meekiana, *Gundlachia* 21: 71
megasoma, *Bulimnaea* 21: 66
megasoma, *Limnaea* 21: 66
Melantho 21: 63
Melongena 21: 14
Menetus 21: 69
mercatoris, *Octopus* 21: 11
Mercenaria 21: 39
mercenaria, *Merecenaria* 21: 39
Micromenetus 21: 69
milesi, *Campeloma* 21: 62
milesii, *Campeloma* 21: 62
Millerelix 21: 99
modicella, *Fossaria* 21: 66
modicella, *Lymnaea* 21: 66
Modiolus 21: 41
montezumensis, *Pyrgulopsis* 21: 82
mooreana, *Daedalochila* 21: 102
Murex 21: 51
Musculus 21: 41
Mya 21: 23
Nassarius 21: 41
neglecta, *Sonorella* 21: 21
neglecta, *Tridopsis* 21: 105
Noetia 21: 39
nolani, *Melantho* 21: 63
nuttaliana, *Limnophysa* 21: 67
obesum, *Campeloma* 21: 62
obrussa, *Fossaria* 21: 66
obrussa, *Galba* 21: 66
obrussa, *Lymnaea* 21: 66
obsoleta, *Ilyanassa* 21: 96
obtusa, *Bithynella* 21: 64
obtuse, *Bythinella* 21: 64
Octopus 21: 11
oleacea, *Physella* 21: 67
Oliva 21: 14
onyx, *Crepidula* 21: 113
orbiculata, *Amnicola* 21: 64
ovalis, *Anadara* 21: 39
pallida, *Amnicola* 21: 64
pallida, *Galba* 21: 66
pallida, *Limnophysa* 21: 66
pallida, *Lymnaea* 21: 66
Paludina 21: 63
palustris, *Galba* 21: 67
palustris, *Limnaea* 21: 67
palustris, *Limnophysa* 21: 67
palustris, *Lymnaea* 21: 67
palustris, *Stagnicola* 21: 67
pansa, *Plicopurpura* 21: 51
parallela, *Ferrissia* 21: 71
parallelus, *Ancylus* 21: 71
parallelus, *Ferrissia* 21: 71
parva, *Amnicola* 21: 64
parva, *Fossaria* 21: 66
parva, *Galba* 21: 66
parva, *Lymnaea* 21: 66
parvus, *Gyraulus* 21: 69
parvus, *Planorbis* 21: 69
Patera 21: 105
pedregosensis, *Sonorella* 21: 18
Pemarginata 21: 64
peregrina, *Daedalochila* 21: 100
peregrina, *Polygyra* 21: 99
perforans, *Crepidula* 21: 115
Physa 21: 36, 67
Physella 21: 67
Physidae 21: 67
Pierosoma 21: 69
Pisidium 21: 35
plana, *Crepidula* 21: 113
Planorbella 21: 36, 69
Planorbidae 21: 69
Planorbis 21: 69
Planorbula 21: 70
Pleurocera 21: 65
Pleuroceridae 21: 65
plicata, *Daedalochila* 21: 100
plicata, *Polygyra* 21: 99
Plicopurpura 21: 51
Polygyra 21: 99
Polygyridae 21: 99
Polygyrinae 21: 99
Polygyrini 21: 99
polymorpha, *Dreissena* 21: 1
Pomatiopsidae 21: 64
Pomatiopsis 21: 64
ponderosa, *Melantho* 21: 63
ponderosa, *Noetia* 21: 39
ponderosa, *Paludina* 21: 63
ponderosa, *Vivipara* 21: 63
ponderosum, *Campeloma* 21: 62
porata, *Amnicola* 21: 64
postelli, *Goniobasis catenaria* 21: 36
Potamopyrgus 21: 78
Praticolella 21: 109
Probythinella 21: 64
producta, *Elliptio* 21: 33
Promenetus 21: 70
Promenetus 21: 71
Pulmonata 21: 102
pumilus, *Ancylus* 21: 71
Purgulopsis 21: 36

Purpura 21: 51
Pyganodon 21: 33
Pyrgulopsis 21: 64, 77
Radix 21: 87
reflexa, *Galba* 21: 67
reflexa, *Limnaea* 21: 67
reflexa, *Limnophysa* 21: 67
reflexa, *Lymnaea* 21: 67
reflexa, *Stagnicola* 21: 67
regulare, *Campeloma* 21: 62
regularis, *Helisoma* 21: 69
regularis, *Paludina* 21: 63
rigida, *Atrina* 21: 12
rivularis, *Ancylus* 21: 71
rivularis, *Ferrissia* 21: 71
rufum, *Campeloma* 21: 62
Runcina 21: 96
sayana, *Amnicola* 21: 64
sayana, *Oliva* 21: 14
sayi, *Physella* 21: 67
sayii, *Physella* 21: 67
scapha, *Anadara* 21: 43
Segmentina 21: 70
senilis, *Anadara* 21: 43
shimekii, *Ferrissia* 21: 71
sincera, *Valvata* 21: 62
sitens, *Sonorella sitens* 21: 22
skinneri, *Physa* 21: 67
Somatogyra 21: 64
Somatogyrus 21: 64
Sonorella 21: 17
Sonorellales 21: 18
Sonorellamorpha 21: 18
splendida, *Lampsilis* 21: 33
stagnalis, *Limnaea* 21: 67
Stagnicola 21: 67
Strombidae 21: 93
Strombus 21: 93
Stylommatophora 21: 102
subcarinata, *Lioplax* 21: 64
subcrenata, *Anadara* 21: 43
subglobosa, *Birgella* 21: 64
subglobosus, *Birgella* 21: 64
subglobosus, *Somatogyrus* 21: 64
subpurpurea, *Viviparia* 21: 62
subpurpureus, *Viviparius* 21: 62
subsolida, *Campeloma* 21: 62
subsolida, *Melantho* 21: 63
subsolida, *Vivipara* 21: 63
subsolidum, *Campeloma* 21: 62
subsolidus, *Campeloma* 21: 63
subulare, *Pleurocera* 21: 65
sulculosa, *Lioplax* 21: 63
tarda, *Ferrissia* 21: 71
tardus, *Ancylus* 21: 71
tardus, *Ferrissia* 21: 71
Telescopium 21: 96
telescopium, *Telescopium* 21: 96
texasiana, *Linisia* 21: 105
Thais 21: 51
Trachycystis 21: 117
transversa, *Anadara* 21: 42
trapezia, *Anadara* 21: 42
tricarinata, *Valvata* 21: 62
Tridopsis 21: 105
trivolvis, *Helisoma* 21: 69
trivolvis, *Pierosoma* 21: 69
trivolvis, *Planorbella* 21: 36, 69
trivolvis, *Planorbis* 21: 69
troostiana, *Daedalochila* 21: 100
troostiana, *Polygyra* 21: 99
truncata, *Helisoma* 21: 69
truncata, *Planorbella* 21: 69
truncatus, *Planorbis* 21: 69
truncatum, *Helisoma* 21: 69
truncatum, *Planorbella* 21: 69
tryoniana, *Sonorella* 21: 21
Tulotoma 21: 83
umbilicata, *Lymnaea* 21: 67
umbilicatellus, *Promenetus* 21: 71
umbilicatelus, *Gyraulus* 21: 71
umbrosa, *Galba* 21: 67
umbrosa, *Limnaea* 21: 67
umbrosa, *Limnea* 21: 67
umbrosa, *Limnophysa* 21: 67
umbrosa, *Stagnicola* 21: 67
umbrosus, *Lymneus* 21: 67
undulata, *Alasmidonta* 21: 33
Uniomerus 21: 33
Unioninae 21: 33
Upsilidon 21: 111
ustulatulina, *Crepidula* 21: 114
utahensis, *Valvata* 21: 77
Utterbackia 21: 23, 33
Valvata 21: 36, 61, 77
Valvatidae 21: 61
vibex, *Nassarius* 21: 41
vibex, *Villosa* 21: 33
Villosa 21: 3, 33, 48
virgata, *Physella* 21: 68
virginica, *Crassostrea* 21: 113
Viviparia 21: 62
Viviparidae 21: 62
Viviparius 21: 62
walkeri, *Physella* 21: 68
watsoni, *Trachycystis* 21: 117
wheatleyi, *Segmentina* 21: 70
williamsi, *Crepidula* 21: 115
zebra, *Limnaea* 21: 67
zebra, *Limnophysa* 21: 67
zebra, *Lymnaea* 21: 67
zonalis, *Batillaria* 21: 96

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Strategies for sustainable dye harvest of the purple conch *Plicopurpura pansa* (Gould, 1853) from west central Mexico. **ERNESTO A. CHÁVEZ and JESÚS E. MICHEL-MORFÍN** 51

The freshwater gastropods of Iowa (1821-1998): Species composition, geographic distributions, and conservation concerns. **TIMOTHY W. STEWART** 59

Experimental studies on habitat preference and tolerances of three species of snails from the Snake River of southern Idaho, U.S.A. **STEVEN LYSNE and PETER KOETSIER** 77

Effects of extracts of the bark of the stem of *Croton tiglium* on the metabolism of the freshwater gastropod *Lymnaea acuminata.* **RAM P. YADAV, D. SINGH, S. K. SINGH, and A. SINGH** 87

Histology of selected regions of the alimentary system of *Strombus gigas* Linnaeus, 1758 (Caenogastropoda: Strombidae). **OMAR H. AVILA-POVEDA, DALILA ALDANA-ARANDA, and ERICK R. BAQUEIRO-CÁRDENAS** 93

Daedalochila sp. nov. from northwest Arkansas, U.S.A., the anatomy of the *Polygyra plicata* group, and the validity of the genus *Millerelix* Pratt, 1981 (Gastropoda: Pulmonata: Polygyridae). **BRIAN F. COLES and GERALD E. WALSH** 99

Research Note 113

Book Review 117

Meeting Announcement 119

Financial Report 121